

nature

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Ask the young about the future

THE House of Commons Subcommittee's examination of the future of scientific research in British universities seems in imminent danger of falling apart—not through any violent centrifugal tendencies of its members, but because a growing sense of boredom is beginning to surround its missions. This past week, Sir Sam Edwards, Chairman of the Science Research Council, and Sir Fred Stewart, Chairman of the Advisory Board for the Research Councils, two of the men most central to policy-making in science, dourly defended the present system against some tepid questioning, and once more one wondered why we were all there.

Part of the reason for the lack of enthusiasm is that everyone knows that the education and science sector is suffering heavily from the ravages of inflation, and it is thus no time for reasonable men to start bickering among themselves. And the Rothschild reorganisations are too recent in people's memories for there to be much interest in another round of fundamental changes.

So what can the committee profitably do? It could switch its attention to the young students who are potentially a driving force in science in the 1980s. Two questions about them are surely worth extended investigation. First, are they going to continue to desert the physical sciences in large numbers? And, second, what in the educational system leads to the alienation between pure and applied science, which is an undoubted contributor to Britain's poor performance in converting research into development and production? The committee has shown more than a passing interest in this latter issue and might find it a more congenial question, and certainly one with major economic implications. Fiddling with the dual-support system and the structure of the Science Research Council can at most have marginal benefits in comparison, although a detailed look at the alienation problem might well lead, in the longer term, to the fundamental changes which nobody seems keen to contemplate at present.

Is there money still for tea?

IN recent months, the Department of Energy has been stepping up its campaign to persuade the British public to save energy. "It's everybody's baby. The man who drives alone to work. The boiler-minder who could get more out of his boiler. The housewife who lets her kettle boil away while she chats on the phone. Just by being careful, you can save your own money—and millions for Britain." And in another advertisement a kettle is shown with pound notes steaming out its spout.

Our back-of-the-envelope calculations say this. Britons drink some 2×10^8 cups of tea or coffee daily, of which half come from kettles, each kettle producing two cups, say. Of these 5×10^7 kettles, perhaps half have whistles or automatic cut-outs. Further, of the remainder only about 10% are kept boiling for any substantial period—let us say on average one minute. Thus British kettles boil unnecessarily for 2.5×10^6 minutes daily. This wastes roughly 10^5 kilowatt-hours of energy every day. This costs the foolish housewives of Britain £1,500 a day or an average of 0.015p per family.

Hardly pounds streaming out of each kettle, you may say, but worth saving nevertheless. But it costs money and it uses up energy to run a publicity campaign. Advertisers measure success at the fractions of a percent level—a campaign that persuaded 1% of the population to change its ways would

have been remarkably successful. At stake are thus only 10^3 kilowatt-hours of energy or the colossal sum of £15 a day—although the steam heats up the room and thus for at least half of the year might be deemed a good thing.

We haven't dared ask the department how much this particular advertisement has cost but it must be several thousand pounds—and in the process energy has been consumed in printing it, distributing it and raising it on to a placard.

Ironically, really worthwhile rather than trifling savings are possible when boiling a kettle. Few measure the water into the kettle and as a result an enormous amount of water is raised to the boil and then not used. It is left as an exercise for the reader to determine how much the department could have saved—then to devise an appropriate slogan.

Similar arguments can also be applied to domestic hot-water systems. Lagging a hot-water tank is fine, and the Department of Energy rightly points out that considerable savings of money and energy can be made in this way, but how many people heat their domestic hot water for unnecessarily long periods in the summer, and indeed how many people have hot-water tanks that are too big for their requirements?



international news

Exit Dixy, with acrimony

by Colin Norman, Washington

DR Dixy Lee Ray, the controversial and widely-respected former chairman of the Atomic Energy Commission (AEC) last week quit her job as the top science official in the State Department because she had been virtually frozen out of foreign policy decisions, even on matters involving research and development. Her letter of resignation to President Ford, which she subsequently made public, also contained a sharp attack on the Administration's domestic science policies, suggesting that "pitifully little is being done" in several critical areas, including the setting of policy for energy research and development.

Ray's appointment to the State Department job was widely regarded as a sign that the department's science and technology bureau would be elevated to an important position in the departmental hierarchy. But, after five months of being ignored, she handed in her notice on June 20, and aired her grievances in a series of television interviews and in an appearance before a Senate subcommittee. Her complaints paint a stark portrait of bureaucratic warfare in the State Department, and a gloomy picture of the manner in which foreign policy involving science and technology is made.

Appointed chairman of the Atomic Energy Commission by former President Nixon in 1973, Ray's appearance in Washington was greeted with reams of prose concerning her "sensible" clothes, her knee socks, her two dogs which shared her office, and her motorized home in which she lived close by the AEC's headquarters. In a lacklustre Administration, she stood out as a colourful character, but she also quickly established a solid reputation as a forceful administrator of one of the most controversial agencies in the federal government. She forced the resignation of several AEC officials, made the agency much more open and responsive to public concerns, and adopted a tough, no-nonsense approach in her dealings with other agencies and with Congress.

When the AEC was abolished by an



Dixy Lee Ray: gone but not forgotten

Act of Congress which established the Energy Research and Development Administration and the Nuclear Regulatory Commission, Ray accepted the job as head of a new bureau in the State Department, with the cumbersome title of Bureau for Oceans and International Environmental and Scientific Affairs (OES). The Bureau was foisted on the State Department by Congress, in a bill passed in 1973, but which was not put into effect by the department for more than a year—a fact which indicated the importance which Secretary of State Henry Kissinger attached to the matter. The new bureau is an amalgamation of several former offices; it was supposed to inject high-level analysis into policies concerning science and technology—such as export of nuclear materials, the world food shortage, ocean research and so on.

Kissinger is, however, well-known for ignoring the State Department's bureaucratic machinery in formulating foreign policies, and relying instead on advice from a few key associates. Ray was never one of those associates, and she said last week that by the time her bureau became involved, policies were already established. The bureau, for

example, played no role in negotiations concerning the controversial agreement between West Germany and Brazil on the sale of nuclear materials, matters concerned with the Law of the Sea Conference are handled by another office, and the prime focus of energy policymaking in the State Department is in the Office of Economic and Business Affairs, which is headed by Thomas O. Enders, a close associate of Kissinger's.

In her letter to Kissinger, Ray said that "for some time I had hoped that my office and the Bureau I head would play a significant role in the formulation of the Department's science policy . . . unfortunately, that desirable condition has not been fulfilled. Many of the areas for which OES has statutory responsibility are, in fact, being pursued in other bureaux and offices." Ray subsequently told the Senate subcommittee on oceans and international environment, which was chiefly responsible for the bill establishing OES, that she hadn't seen Kissinger once since she was appointed. She had asked for a meeting to discuss her concerns, she said, but was told to prepare a "briefing paper" instead.

In a longer letter to President Ford,

Ray said "the (OES) Bureau can do little but acquiesce in the policies set by others, and attempt to implement its broad responsibilities with little authority and few resources". She added that "similar problems plague our Nation's domestic science policy ... I am deeply concerned that the imperative to use existing and proven technology for vigorous attack on today's problems is not fully recognised nor appreciated at the highest levels of government."

Ray went on to point out "pitifully little is being done" in the area of energy resources and technology. "Our country is drifting", she said, "we seem neither to have the will to conserve energy nor the courage to map out a national program that will free us from the bondage of too great a reliance on imported energy".

After delivering herself of her grievances, Ray departed last Friday to her native Washington State in her motor home, to complete a book aptly titled "Good-bye America". Washington DC will miss her.

● SUMMER has settled in on Washington, bringing with it mind-boggling allegations of wrongdoings by the CIA, utter indecision in Congress over energy policy, growing speculation about next year's presidential elections, and a smog level officially deemed hazardous to health. Against that background, the mixed political fortunes of the National Science Foundation (NSF) haven't been accorded much attention by the daily press, but last week the House of Representatives took a couple of significant votes on NSF's activities.

The House approved an appropriations bill which would slash \$44.3 million from the Ford Administration's proposed budget for NSF for the financial year which began on July 1, ensure that basic research would bear the brunt of that cut, and stop the foundation promoting the use of school science courses. The bill now goes to the Senate, where its prospects are uncertain, but whatever the Senate decides, the hostility toward NSF so far demonstrated by the House is cause for some concern.

The prohibition against promoting school science courses is particularly noteworthy. The NSF has for years been developing innovative school science curricula, which it has promoted by sponsoring courses for teachers to acquaint them with the new materials. But this practice ran into right-wing criticism in the House earlier this year over an NSF-sponsored course called MACOS (Man: A Course of Study). That criticism, in fact, led to many of NSF's other political problems.

Briefly, a number of conservatives in the House attacked MACOS because

it depicts the rough realities of Eskimo life rather explicitly in a number of films and books. The leading critic, Representative John B. Conlan, a Republican from Arizona, has described the course as undermining traditional American values, and he objected to the fact that NSF was promoting it against other, privately developed, courses. Conlan proposed an amendment to an NSF bill in April to stop NSF promoting school science courses because, he said, such activities are leading government control of the content of local education. His amendment was narrowly defeated, but the furore which ensued led the House Committee on Science and Technology, and the National Science Foundation itself, to conduct a thorough review of NSF's education programmes.



In spite of the fact that Conlan's amendment was defeated, the House Appropriations Committee approved a budget bill for the foundation on June 20 which expressly forbids NSF from spending any money on promoting school science courses. The committee noted that MACOS is now being taught in 1700 schools throughout the United States, and suggested that use of government funding for promoting particular courses gives them an unfair market advantage. The House adopted the committee's recommendation with scarcely any debate.

The irony in all this is that Congress has in the past been critical of NSF for doing too little to implement the courses, research findings and so on, which result from activities it has funded. Moreover, many educators have pointed out that school science education was in a mess before NSF arrived on the scene.

As for other parts of the NSF appropriations bill, the House decided that NSF should have \$727 million to spend in the 1976 fiscal year (including \$20 million in funds carried over from 1975), which is \$44.3 million less than the Administration requested. The House, moreover, suggested that \$35 million of the cut should be taken from the budget proposed for research grants. If accepted by the Senate, the bill would provide a total of \$345 million for research grant support, which is an increase of only \$5 million over last year's level. The House Appropriations Committee, which recommended the reduction, said that this was a

proper level of funding to maintain.

● The second significant vote on NSF taken by the House last week could, however, provide some relief for that beleaguered agency. A House-Senate conference committee was appointed to decide the fate of, among other things, the so-called Bauman amendment to give Congress power to veto individual NSF grants before they are awarded. The amendment had been attached to the House version of the NSF authorisation bill, but it was not approved by the Senate. When the conference committee was appointed, the amendment's sponsor, Robert Bauman, a Republican from Maryland, proposed a resolution instructing House members of the conference committee to ensure that the amendment is written into the final version of the bill. The resolution was defeated by 127 votes to 284, and it is now considered likely that the Conference Committee will water down the amendment.

● The trouble-plagued liquid metal fast breeder reactor (LMFBR) programme, which is the costliest energy research and development effort in the United States, survived a crucial test in Congress last month. A move to delay construction of the proposed LMFBR demonstration plant for 18 months, while the entire programme is evaluated, was defeated in the House of Representatives by a wide margin—136 votes to 227. A similar move is likely to take place in the Senate next week.

The challenge to the breeder programme came during a debate on a budget bill for the Energy Research and Development Administration. An amendment to delete funds for the demonstration plant and to prohibit ERDA from buying capital equipment for it, was proposed by Representative Lawrence Coughlin, a Republican from Pennsylvania, who argued that the programme should be postponed until it is determined whether or not the breeder is needed. The National Academy of Sciences is among the organisations which is looking into the LMFBR programme.

Although the amendment was heavily defeated, opponents of the LMFBR programme are not too despondent. A year ago, they point out, such a move would probably have gathered only a handful of votes, but unease over some aspects of the LMFBR programme, including its projected cost of \$10.7 billion, caused nearly a third of the House to vote in favour of a delay. It is, nevertheless, generally agreed that this is the critical year for the LMFBR programme. If Congress now sanctions large expenditures on the LMFBR demonstration plant, that investment will make it difficult to turn the programme off in later years. □

A CONTEMPORARY odyssey of pain, repression and, finally, of achievement, reached its climax this week at a ceremony on the Mount Scopus campus of Jerusalem's Hebrew University, in which an outstanding 26-year-old mathematician was awarded a PhD and the prestigious Aharon Katzir Prize. It began six years ago in the town square of Riga, Latvia, where that same young man, Ilya Rips, set himself on fire in order to dramatise his opposition to the Red Army invasion of Czechoslovakia and to Soviet anti-Semitism. Fortunately, passers-by snuffed out the flames and rushed him to a hospital.

Rips was subsequently sent to join other dissidents at a mental institution, where he spent over two years "under treatment". Had it not been for wave after wave of international protests, he would probably still be there. From the time he arrived here in January 1972, until this week, Rips—a naturally very reticent person—stayed out of the headlines. He was content to learn Hebrew, which he now speaks with great fluency, and then to immerse himself once again in the world of mathematics, where his work on the dimensional sub-group problem, begun in Riga, has already aroused international interest. Now he is to be a lecturer in the Hebrew University's Mathematics Department.

● Dozens of government personalities were at the Hebrew University ceremony, but few of them have any idea what is really going on in the lecture rooms and laboratories of local institutions of higher learning. Alarmed at the possible consequences of this situation, Israel's academic centres have taken it upon themselves "to educate" the country's policy-making elite. At the Weizmann Institute alone, day-long seminars on various aspects of science have recently been held for members of the Knesset (Israel's Parliament) and for senior officials of the Ministries of Finance, Commerce and Industry, Foreign Affairs and Education. Although the participants sometimes seem to be slightly bewildered by talk about polymers, antigens and quarks, they generally manage to retain their perspective. For example, when a Weizmann Institute official greeted a group of Finance Ministry officials, he earnestly explained to them that the seminar was strictly educational in character and was not being held "to sell you the institute".

"Don't worry," a Finance Ministry man replied, "we don't have the money to buy it. But perhaps you'll give us an option on the place."

● Government monetary limitations

notwithstanding, official bodies are putting more money into the development of solar energy, which, as Minister of Commerce and Industry Haim Bar-Lev recently stated, they hope will give the country increased 'economic energy'.

Twenty years ago Israel was in the forefront of solar energy research, but interest in the subject rapidly declined here, as elsewhere, when low petroleum prices seemed to obviate any need for what was then a much more expensive form of energy. Nevertheless, Israel is probably at present the world's largest

Letter from Israel

from Nechemia Meyers

user, per capita, of applied solar energy.

Backing up this contention in a paper presented at a recent solar energy conference in Rehovot, Dr Harry Tabor pointed out that:

(1) The Dead Sea Works, with approximately 100 km² of evaporation ponds, use solar heat equivalent to about 30 × 10⁶ tons of fuel oil per year—three times Israel's annual national consumption.

(2) Over 100,000 domestic water heaters (which save about 100,000 tons of fuel oil that would otherwise be required for heating water) represent about 0.1 m² of flat-plate solar collector per capita.

Tabor sees justification for using solar energy more widely to heat water, to heat and cool buildings and even to provide a power source for desalination. He argues that if solar ponds could be built for less than \$10 per m²—which seems feasible—it would be cheaper to use solar energy for desalination than fuel costing \$80 a ton.

● Whatever may be the value of Israeli solar energy research, local agricultural research proves its economic significance year after year. Just recently, for example, scientists at the Hebrew University's Faculty of Agriculture reported on the development of new techniques which should facilitate overseas sales of two important export crops—citrus and flowers.

Confronted with the fact that oranges grown north or south of Israel's traditional Coastal Plain citrus belt tend to have rough peels, which makes them unsaleable in Europe, Shaul Monselise, Raphael Goren and Yair Erner, of the Hebrew University, found a way 'to smooth the peels' with a synthetic growth retardant.

Peel roughness is caused by excess levels of gibberellic acid and cytokinins, hormones which control cell division and cell enlargement in the

oranges (as they do in other plants). Since there is an excess of these growth regulators in the early stages of the fruit's growth, the team found that peel roughness could be controlled by spraying Alar (a growth retardant) on the citrus during that period. This cut down roughness by 30% without affecting the fruit's nutritional value or making it unsafe to eat. Now equal success is being achieved with a less expensive chemical (CCC).

● Israeli agricultural research is distinguished not only by its quality, but also by the fact that it reaches the farmers and the fields much more quickly than it would in other countries, even those with well organised extension services. This is partly because one-third of Israel's agricultural students are themselves settlement members, who rush back home with any promising new hybrid or hormone. In addition, local farmers are not content to wait quietly for solutions to their problems; instead, they keep pestering the professors for assistance and, moreover, they are willing to try innovative methods even when they are still experimental.

Agricultural know-how reaches Arab farmers almost as quickly as it reaches the members of Jewish settlements. The 17 Arab students at present enrolled at the Hebrew University's Faculty of Agriculture, as well as Arab graduates, bring new methods to their home villages (inside the country's 1967 borders) and to the West Bank and the Gaza Strip. Production has risen spectacularly in these latter areas since Israeli troops, closely followed by Israeli agricultural advisers, entered them eight years ago.

This rapid transfer of new forms of cultivation, new seeds, new fertilisers and new pesticides could pose a problem for Jewish farmers, because it deprives them of the technological advantage which they would otherwise have over Arab farmers. Indeed, certain crops that were once grown mainly by Jewish farmers, such as strawberries, have been taken over almost completely by Arab farmers, who have access to the same agricultural research and, at the same time, spend less on manpower because their own large families provide a bountiful source of practically free labour.

Yet the Dean of the Hebrew University's Agricultural Faculty, Professor Isaac Harpaz, is not unduly worried. He points out that the economies of Israel and the Administered Areas are closely linked, and likely to remain so whatever form of political settlement is eventually achieved. Thus prosperity for Arab farmers benefits the entire economy.

THERE is a good deal of confusion at the Pasteur where a lively campaign is being waged, by means of handbills, rumours and articles in the press, against the genetic manipulation experiments which have just started there.

The story begins a year ago; indeed when the Asilomar Conference of February 1975 gave the go-ahead to the genetic manipulation experiments which had been temporarily halted, French biologists who wished to do similar research were hardly taken unawares. As early as November 1974, they had asked first the CNRS and then the Délégation Générale à la Recherche Scientifique et Technique to set up an organisation to exercise some form of control over research which nobody denies could possibly be dangerous. Two committees were formed; one, with the task of ruling on the ethical problems arising from the experiments, was chaired by J. Bernard, who is Director of the Research Institute for Diseases of the Blood in Paris. It is made up of J. Monod, F. Jacob, F. Gros, R. Monier, J. P. Ebel, Y. A. Chabert and P. Slonimsky.

The other is a committee of experts comprising 15 researchers, doctors and biologists. With the final document of

the Asilomar conference as a guideline, they defined the safety limits to which the experiments submitted to them must conform. The parent organisation (CNRS, INSERM, etc.) would then be responsible for the effective control of the experiments. In this way, for example, a subcommission on hygiene and safety will soon be set up at the Pasteur.

Asilomar and the Pasteur Institute

from the staff of La Recherche

At the same time, the DGRST gave a grant of 300,000F to build a laboratory to house 'the moderate risk' experiments in the Pasteur. It was there that the so called 'low risk' experiments were initiated—those which had not been delayed in order to wait the Asilomar conclusions; and it was there that the 'moderate risk' experiments were to be started which could result in new vaccines.

The internal meetings at the Pasteur were to inform the whole staff, both researchers and technicians, of the

results of the Asilomar conference and of the experiments which were to be carried out.

Nobody expected the explosion which followed these meetings, the first of which had taken place in April. One group of research workers and technicians undertook to fight both the decision to place the special laboratory within the department of molecular biology and also the experiments themselves. An atmosphere of panic overran the Pasteur. Institute biologists and others formed themselves into a 'biological-information group' and recently they have published a manifesto demanding the suspension of experiments and a public meeting where the advantages and disadvantages of the experiments could be debated.

The dissenting movement is very large. It has to be seen within the political and philosophical framework which brings into question genetic manipulation as well as other biological research, virology, genetics etc., where the dangers must be weighed against expected benefits. The movement contests the present reliance on the scientific expert. It questions the way in which science is run, both at the Pasteur, and in general. And finally it poses the problem for whom is science done, scientist or people?

A NUMBER of choices and decisions must be taken this month and next to rationalise international effort on energy research and development, which, otherwise, promises to be a farce or to duplicate what is being pursued nationally in several places. Perhaps the most significant of these moves is the one Dr Walter Marshall, Chief Scientist at Britain's Department of Energy (and Director of Harwell) claims as his personal responsibility. This is a procedure available within the 1974 Kissinger-initiated International Energy Agency (IEA) for developing commercially valuable products on a consortium basis and for protecting these by licencing.

Marshall points out that it is easy to collaborate internationally on basic research and on the exchange of results, but very difficult to do work of commercial value where governments are involved, particularly the US government. This is first, because of the difficulty of interlocking government management, industry and government laboratories. International schemes which involve the US federal government, for example, fall foul of the American system whereby all information derived through federal participation must be freely shared with American industry. Some months ago Marshall successfully argued that commercial undertakings should be in-

Marshalling our power

from Angela Croome

cluded in the agency's programme and that industrial protection was needed for non-American industrial participants.

Only 10 days ago a compromise agreement was reached after several midnight sessions and this has been recommended to the British government for endorsement. It provides a means of setting up industrial consortia with defined objectives and the production of industrial 'products' for licencing. Member countries of the IEA can opt out of any of them. When the product has emerged the countries which had not chosen to join in that particular project would have either to buy a licence to manufacture or make a down payment later.

Nine research themes for the IEA have been chosen so far, including coal, energy from municipal and industrial waste, radioactive waste management, fusion use of waste heat, energy conservation, solar energy, hydrogen conversion and nuclear safety. The concept of the 'lead country' is, in the main, being adopted in the organisation of the work, existing investment and effort being the leadership criteria.

Of the programmes already well developed, for example, fusion work is likely to be led from within the EEC but application is still too far off for a commercial consortium to be needed.

Britain may well take the leadership on coal utilisation research and the fluidised bed development has now reached the consortium stage. Britain has also put money into its own radioactive waste programme so would be expected to participate in the agency's.

Many of the IEA themes are duplicated under the EEC's joint programme, but the organisation and decision-making structure of the latter is very different. The requirement that all EEC decisions have to be unanimous results in decisions being political rather than scientifically based. Whatever may be the point of view of the advisers of other EEC countries, Marshall expects to get better value out of the IEA. The budget for Europe's Joint Research Centre is still under review, though expected to be settled this month, and there is every likelihood that most of the proposed programme will be adopted and for this there are obligatory contributions. For EEC members such as Britain, therefore, it seems logical for the IEA to subsume many of the same programmes. By routing participation through the EEC, member countries should avoid paying twice. □

Weather wise

from Vera Rich

THE recent talks in Geneva between Soviet and US delegations on the banning of climatic manipulation for military purposes has focused attention, once again, on the draft convention dealing with weather warfare which the Soviet Union presented to the United Nations last autumn, and which, at the time, was viewed in many quarters as little more than a kind of appendix to a general concern with problems of the environment.

Russia has, of course, a long history of climatic warfare, and her secret allies 'General January' and 'Field-Marshal February' have, through the ages, proved the downfall of Swedes, Poles, French and Germans. In a situation of conventional warfare, the Russian army has only to retreat sufficiently far and sufficiently slowly for the invader to be trapped by winter—and the climate does the rest. But, endowed as she is with a natural

strategic weather advantage, the Soviet Union has been quick to see the possibilities of artificial climatic manipulation. Recent articles in *Mezhdunarodnaya Zhizn'* and *Krasnaya Zvezda* have drawn attention to a number of possible artificial meteorological weapons, ranging from the melting of the Arctic ice and tsunami (tidal waves) caused by nuclear explosions on the edge of the continental shelf to the use of infrasound and atmospheric activity to produce psychotropic effects—depression, terror, and the "suppression of mental activity"—in large groups of the enemy in specific target areas. According to the articles in *Mezhdunarodnaya Zhizn'*, the Americans are researching the possibility of "changing the nature of lightning", with a view to being able to direct electrical charges of "tremendous" power against specific targets.

It would be interesting to know the source of this last piece of information. When Academician Kirillin, the Soviet Minister of Power, visited Britain in May 1974, he was shown an impressive

experiment at the Culham Atomic Energy Research Establishment in which lightning striking an aircraft in flight was simulated. Could this have been interpreted as a front for some sinister NATO—and therefore, in Soviet eyes, American—weapon?

Some of the other potential weapons detailed in the Soviet press verge on the fantastic—a localised window in the ozone layer to admit ultraviolet radiation, for example—and it seems difficult to see why Mr Brezhnev has found it necessary to stress the urgency of new agreements banning development in this field. Perhaps the urgency is more a matter of politics than immediate military danger. As a preliminary to the projected Ford-Brezhnev summit meeting, it would be gratifying if the USA and the USSR could reach some form of international agreement in the cause of peace and friendship. And what could be easier than for both sides to renounce the use of a weapon that neither actually possesses at the present time. □

THEOLOGY was once known as "the queen of the sciences", and although an examination of recent *Nature* indexes does not produce many references to this subject, I think it may be permissible to mention the idea of the "God of the gaps". I have unfortunately been unable to trace the origin of this phrase, which is used to describe the views of those who are unable to come out clearly as rationalistic atheists. They hang on to the idea of a deity, but restrict his activities to the gaps between the major fields of man's activities, where science is thought to be able to give a full explanation.

My interest in this theological proposition was aroused when I realised that it was very similar to the view held by many leading conservationists. They say that they believe that wildlife and countryside preservation is important, but they acknowledge that the interests of the farmer and of food production must have priority in any scheme for managing the rural landscape. This point is explicit in the recent report of the Countryside Commission, *New Agricultural Landscapes*. Any wish to retain the familiar pattern of hedges, picturesque buildings and flower-rich though rather unproductive meadows is castigated as sentimental. We are urged to try to make the best of the inevitable.

It must be admitted that, even within this pattern of farming development, useful compromises which have done much to spare wildlife (particularly birds) have been possible. There have now been many 'Silsoe-type' exercises, for example. These follow the pattern

Don't ditch hedging



KENNETH MELLANBY

of the first weekend conference at Silsoe in Bedfordshire, when agriculturalists and conservationists tried to work out various schemes for managing a farm where nearly maximum productivity could be married with the least damage to the native flora and fauna. Small, unproductive patches on the farm are identified, and these are planted with trees and shrubs to act as mini-nature reserves.

All these developments result, however, in some impoverishment of the landscape and a reduction in numbers of many native plants and animals. This is accepted because of the belief that we must do everything possible to maximise food production, as otherwise we may all face malnutrition at the best and mass starvation at the worst.

A country like Britain that imports nearly half its food cannot enjoy the luxury of 'wasting' any area for conservation if it can be used for food production.

I believe that the time has come for conservationists to be much more aggressive. Farmers have a right to make a reasonably good living from what may be a very difficult and exhausting job. The government has the duty to see that Britain's food supply is safeguarded, even if the pound sterling falls in value so that we cannot continue to import so much of what we eat. There is a good chance that in a few years the growing world population will absorb any surpluses, and that we will be unable to make good food deficiencies by imports. All these points are taken as arguments in favour of maximising productivity and treating conservation with caution.

I do not believe, however, that the choice is really between the risk of starvation with a rich and varied countryside, and enough food with an impoverished landscape. Britain already produces enough food to provide its population with an adequate diet, with enough calories and protein for all. Imports are mainly used to feed livestock to provide meat, which is produced for enjoyment rather than to prevent any malnutrition. The choice is really between two forms of enjoyment—a meat-rich diet or a countryside rich in wildlife. So the conservationist need no longer be content with the gaps—he can come out in to the open and rightly demand his share in shaping the future pattern of all parts of the rural landscape.

news and views

Viruses and histocompatibility antigens: an unexpected interaction

from E. Lennox

In the minds and activities of immunologists and immunogeneticists those highly polymorphic cell membrane proteins, the major histocompatibility antigens, loom large. These proteins and the products of other genes closely linked to the ones that specify them dominate a wide range of immunological phenomena, among them reactions to tissue transplanted from one individual to another and reactions in culture of mixtures of lymphocytes from different individuals.

In fact thymus-derived cells in particular seem to react strongly and with high frequency to histocompatibility antigens of other members of the species (for example Howard and Wilson, *J. exp. Med.*, **140**, 660; 1974), and even to those of other species (Lindahl and Bach, *Nature*, **254**, 607; 1975). But transplantation and the mixing of lymphocytes in culture are devices of the experimentalist. To what natural phenomena do they correspond? How would the cells of one individual know the histocompatibility antigens of another? Explanations have been sought in phenomena that do lead to cell mixing: fertilisation and gestation (in mammals, at least), sorting during differentiation, transmission of cancer cells (see Bodmer, *Nature*, **237**, 139; 1972; Burnet, *Nature*, **245**, 359; 1973) but none have rung the bell of general acceptance. In fact it can be argued (for example, Cohn, in *Genetic Control of Immune Responsiveness*, edit. by McDevitt and Landy, 427; Academic, New York and London, 1972, and Lafferty and Cunningham, *Aust. J. exp. Biol. med. Sci.*, **53**, 27, 1975) that the apparent importance of the major histocompatibility antigens is exaggerated by the peculiar relation to the immune response and that other polymorphisms are probably equally important.

Now, a new element has been added: in an infected animal, virus-infected cells are vigorously attacked by thymus-derived lymphocytes and the antigen they see is a major histocompatibility antigen modified in a way specific to each virus. Since, moreover, viruses do travel from one individual to another, it is natural to seek an explanation for

histocompatibility antigen polymorphism in terms of this remarkable phenomenon (Doherty and Zinkernagel, this issue of *Nature*, page 50).

In their first reported experiments with lymphocytic choriomeningitis (LCM) virus (Zinkernagel and Doherty, *Nature*, **248**, 701; 1974) injected into mice of different histocompatibility types (*H-2* type) they detected cytotoxic T lymphocytes that would kill only LCM-infected cells of the same *H-2* type as the donor of the cytotoxic lymphocytes. This suggested two possible explanations: either cytotoxic lymphocytes, in order to kill, had to match their *H-2* with that of the target or the antigen to which the lymphocytes were sensitised is a virus-induced modification of the histocompatibility antigen of the cell target. Experiments by them and others indicate that the latter is the more likely.

About the same time Blanden (in *Progress in Immunology*, **2**, vol. 4, edit. by Brent and Holborow, 117; Associated Scientific, Amsterdam and New York, 1974; and Gardner, Bown and Blanden, *Eur. J. Immun.*, **5**, 122; 1975) reported similar findings with ectromelia virus in mice and had shown earlier that the new antigen produced by LCM and by ectromelia in cells of the same histotype were distinct. The total lack of relationship between LCM and ectromelia makes their similar effects on *H-2* antigens even more striking. Further experiments have defined more precisely the requirements for *H-2* matching of the virus-infected generator of cytotoxic lymphocytes and of the target (Blanden *et al.*, *Nature*, **254**, 269; 1975).

By using mice of strains with recombinant *H-2* haplotypes on the same genetic background, they were able to show for LCM- and ectromelia-infected mice that *K* or *D* end matching of the generator of the cytotoxic lymphocytes and of the virus-infected target sufficed. This confirmed their previous findings that *M* locus and minor histocompatibility differences were unimportant. Of special interest was the observation that matching of the *I* region (the region of the immune response genes and of the *I* antigens)

was not essential but may exert a minor effect. This tends to emphasise a different kind of mechanism than the *H-2* region matching required for T cell help of B cells for in this case *I* region matching is necessary (Katz *et al.*, *J. exp. Med.*, **141**, 263; 1975).

There have been attempts to elucidate the mechanisms by which viral infection generates a new specificity that depends on the particular virus and the haplotype of the infected cell. Experiments on a seemingly unrelated system provide clues. Shearer (*Eur. J. Immun.*, **4**, 527; 1974) had shown that coupling of the trinitrophenyl group (TNP) to the surface of spleen cells made them good inducers of cytotoxic lymphocytes when cultured with spleen of syngenic mice. Moreover, the major cytotoxicity was directed not at TNP itself (see also Dennert, *Nature*, **255**, 712; 1975), but at the histocompatibility antigen, modified by reaction with TNP. In the virus-infected cell one might imagine an interaction of *H-2* protein with viral protein to induce appearance of previously unrevealed determinants in a way that is specific for the *H-2* protein and the viral protein.

In favour of this mechanism are several observations. First, the amount of normal *H-2* on ectromelia-infected cells seems reduced, for they are less sensitive to killing by lymphocytes specific for their unmodified *H-2* (Gardner *et al.*, *Eur. J. Immun.*, **5**, 122; 1975). Second, with vaccinia-infected cells and cytotoxic lymphocytes from vaccinia-infected mice, anti-*H-2* serum acting on the target will block cytotoxicity (Koszinowski and Ertl, *Nature*, **255**, 552; 1975). Needless to say, similar *H-2* matching of the generators of cytotoxic lymphocytes and of target cells are required as for LCM and ectromelia (Koszinowski and Thomssen, *Eur. J. Immun.*, **5**, 245; 1975). In addition, Koszinowski and Ertl show that anti-vaccinia serum, not cross reacting with *H-2*, also blocks. Finally, the amount of *H-2*, measured by absorption, is reduced on infected cells and preincubation of them with anti-*H-2* reduced the amount of anti-vaccinia that could bind. All this

indicates a close physical relationship between H-2 and the viral protein that might induce the H-2 protein to reveal new determinants and hide old ones.

Now we are back full circle. What can all this have to do with histocompatibility antigen polymorphism? Doherty and Zinkernagel (page 50) attempt to tie it to a mechanism for enhanced resistance to viruses. They suggest that the assured heterozygosity at the two ends of the *H-2* locus in a random breeding population gives the virus four different proteins to modify, two at the *K* end and two at the *D* end. This would induce a more vigorous immune response. In support of this they compare the lytic activity of lymphocytes of LCM-infected CBA mice (*H-2^k*) on cells of:

$$\begin{array}{l} \text{type 1: } \frac{K^k D^k}{K^k D^k}; \\ \text{type 2: } \frac{K^k D^k}{K^b D^b}; \\ \text{type 3: } \frac{K^k D^d}{K^k D^d} \end{array}$$

Compared to the activity on homozygous *k* cells (type 1), the activity on heterozygous *k/b* (type 2) is reduced as it is on the homozygous recombinant *k/d* (type 3). Moreover F1 mice infected with LCM show stronger immune responses than either parental strain.

It is easy to flaw their proposal either on grounds of plausibility or experimental details. The effects they measure on cytotoxicity are not large and in fact that measured on the heterozygous target and on the homozygous recombinant differ as much from each other as from that on the homozygous parent. In the *in vivo* experiments, the parental strains are not congenic, thus many other factors might be involved in the increased response of the F1. Indeed Koszinowski and Ertl show with vaccinia that F1 mice generate more cytotoxicity when measured on cells of either parent than do the parental strains themselves. In any case, for the argument of Doherty and Zinkernagel, LCM is an unfortunate choice. In this case the augmented immune response in F1 mice leads to quicker death and higher lethality for a given virus dose. This may not be true for all viruses of course (for example Blanden, *Transplant Rev.*, **19**, 56; 1974) but it does cool the fervour of the embrace of their proposal.

From the point of view of plausibility, theirs seems a weak argument for polymorphism. In the first place, the animal already has several viral proteins to respond to. In addition even with the mechanism of modifying self proteins, there are many other ways to increase

the number of surface proteins available for modification without making them polymorphic. Simple repeated duplications would do. It is true these duplications might drift, but there would be no strong demand for new polymorphisms.

Nonetheless, theirs is an attractive idea and an extension of their proposals might bring histocompatibility polymorphism into a central position. Suppose we return to the observations that, for reasons still unknown, T cells respond with high frequency and vigour to other histocompatibility antigens. If we further imagine that interactions with viral protein makes one histocompatibility protein look like some other member of the set, then the apparent preoccupation of T cells with antigens of this set could be put to good use for controlling viral infection. Polymorphism would be advantageous

if the special relation to the immune system were maintained for all variants, and if there were repeats in the set of hidden determinants revealed by interaction of the virus with the polymorphic proteins. Otherwise every new histocompatibility antigen variant in interaction with a virus would give determinants outside the set. In sum, this proposal is a specific form of 'molecular mimicry' of histocompatibility antigens by viral proteins (Snell, *Folia biol., Praha*, **14**, 335; 1968).

And if tumour-specific antigens of chemically induced sarcomas were also members of the same set of histocompatibility antigens as recent experiments indicate they might be (Invernizzi and Parmiani, *Nature*, **254**, 713; 1975) then we might be even closer to understanding polymorphic histocompatibility antigens and the apparent preoccupation of the immune system with them.

Morphine and cyclic nucleotides

from L. L. Iversen

UNDERSTANDING the mechanism of action of morphine and other opiates, and the phenomena of tolerance and physical dependence associated with their repeated use, has been for many years one of the major unsolved problems in neuropharmacology. Important progress, however, is now being made in this area. A major breakthrough was the development of biochemical methods for detecting receptors in brain and other tissues, based on the finding that a specific binding of radioactively labelled morphine-like drugs such as etorphine, or morphine antagonists, such as naloxone, could be detected in homogenates of brain (Pert and Snyder, *Science*, **179**, 1011; 1973; Tirenus, *Acta Pharmac. Tox.*, **32**, 317; 1973; Simon *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1947; 1973; see review by Snyder, *Nature*, in the press). Binding experiments of this type have greatly facilitated studies of the precise anatomical distribution of morphine receptors in the brain, and have helped to elucidate the interactions between morphine-like drugs and morphine antagonists and their receptors.

In a search for a simpler model system in which to study opiate effects, a series of tumour cell lines derived from the C1300 neuroblastoma has been screened for the presence of specific opiate binding sites, using labelled naloxone binding. Although specific binding sites could be detected in several cell lines, a hybrid neuroblastoma × glioma line, NG108-15 (also known as 108CC15) was found to have a particularly high density of morphine

receptors (Klee and Nirenberg, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3474; 1974).

This cell line has since been used to test the hypothesis suggested by Collier and Roy (*Nature*, **248**, 24; 1974) that morphine acts as an inhibitor of a prostaglandin-stimulated adenylate cyclase mechanism. Collier and Roy showed that morphine and related opiates blocked the increased formation of cyclic AMP caused by addition of prostaglandin *E*₁ (PGE₁) to homogenates of rat brain. This effect seems to be related to the normal pharmacological actions of morphine because it could be blocked by low concentrations of the specific morphine antagonist naloxone, it was mimicked by the pharmacologically active drug levorphanol but not by its inactive stereoisomer dextrorphan, and the rank order of potencies of several morphine-like drugs in the biochemical test system correlated well with their known analgesic potencies (see Roy and Collier, *Life Sciences*, 1975, in the press). Traber, Fischer, Latzin and Hamprecht (*FEBS Lett.*, **49**, 260; 1974 and *Nature*, **253**, 120; 1975) recently reported that similar phenomena could be observed in the hybrid glioma-neuroblastoma cells which possess morphine receptors. In these cells, as in brain homogenates, morphine and related drugs antagonised the increased formation of cyclic AMP evoked by PGE₁ and this effect was stereospecific and blocked by naloxone. Sharma, Nirenberg and Klee (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 590; 1975) made similar observations, and found that the drug concentrations needed for

inhibition of adenylate cyclase by opiate drugs, with or without prostaglandin stimulation, correlated well with the potencies of the same drugs in displacing labelled naloxone from receptor binding sites in these cells.

The results obtained in the hybrid cell line, however, cast some doubt on the view that an interaction of opiates with a prostaglandin-stimulated mechanism is a crucial component in their actions. Sharma *et al.*, for example, found several cultured cell lines that showed increased formation of cyclic AMP in response to PGE₁, but only in those cells that possessed specific binding sites for opiates was this response blocked by morphine. Even in those cells that did respond to morphine the drug also depressed basal adenylate cyclase activity in the absence of added PGE₁, an effect not seen in brain homogenates. Traber *et al.* (1974), furthermore, showed that the interaction between morphine and PGE₁ was non-competitive. All these results suggest that morphine may not act at the same site as PGE₁.

In their most recent paper (this issue of *Nature*, page 57), Gullis, Traber and Hamprecht describe a novel action of opiate drugs in the hybrid glioma-neuroblastoma cells. They found that low concentrations of morphine and levorphanol (but not dextrorphan) stimulated the formation of cyclic GMP, while depressing the concentration of cyclic AMP. These effects were blocked by naloxone, which by itself at low concentrations had no effect on cyclic nucleotide levels. At higher drug concentrations a somewhat complicated picture emerged, since morphine, levorphanol, dextrorphan and naloxone all caused increases in cyclic AMP levels. The latter effects seem unlikely to be associated with the specific pharmacological actions of these drugs. The stimulation of cyclic GMP formation by low concentrations of opiates, however, represents a novel mechanism that could possibly explain the actions of these drugs in reducing cyclic AMP levels and antagonising prostaglandin effects on cyclic AMP, since reciprocal interactions between the two cyclic nucleotides have been found in a variety of other biological situations.

Recent findings by Klee, Sharma and Nirenberg (*Life Sciences*, 1975, in the press) also provide new insight into the processes of tolerance and dependence at a cellular level. They found that on continued exposure of the cultured hybrid cells to morphine the cells adapted by an increased adenylate cyclase activity. The cells thus show 'tolerance' in the sense that their cyclic AMP contents are similar to those found in normal cells, in spite of the continued presence of morphine, and

Crossed lines

from Pamela Hamlyn

SINCE human beings are inclined to choose their own mates and to produce a mere handful of offspring, it is hardly surprising that mapping of their genes by classical methods has not been as successful as that of the much more accommodating fruit fly. The mapping of human genes, that is, locating them on the twenty-four chromosomes, is of particular interest as an approach to the understanding of human diseases which arise from the inheritance of faulty genes. It is therefore very fortunate that other methods exist for gene mapping. Of these, the formation of a hybrid cell between two different cell types is probably the most important.

Recently a group of scientists working at the National Institutes of Health (Bethesda) have described two different hybrid cell lines which they suggest might be useful for the location of globin genes, and in addition possibly provide information on genetic factors involved in the regulation of their transcription (Deisseroth *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1102; 1975).

One of the hybrid cell lines was formed by fusing Friend mouse erythroleukaemia cells with human fibroblasts. The Friend cells grow in culture and synthesise very low levels of haemoglobin but can be stimulated by the addition of dimethylsulphoxide (Me₂SO) so that 20–50% of the cells give a positive reaction for haemoglobin as detected by benzidine staining. The hybrid cells retain most of the chromosomes of both parents but do not synthesise haemoglobin even when Me₂SO is added to the culture medium, although it can be inferred that at least the β -globin gene is retained. This behaviour—the 'extinction' of the characteristic protein of a specialised cell, when that cell is fused with a relatively undifferentiated cell—is a well documented characteristic of this type of cross. In general it is not clear at what site extinction takes place although conjecture favours transcription of the gene. In these experiments a 'probe' of complementary (c) DNA, made by transcribing purified globin mRNA with reverse transcriptase, has shown that globin mRNA is not present in these hybrids, suggesting

that extinction takes place at the level of transcription or mRNA processing.

The other hybrid cell line described was formed by fusing the same line of mouse erythroleukaemia cells with erythroblasts from human bone marrow. Erythroblasts are red blood cell precursors. They contain globin mRNA as detected by a cDNA probe and haemoglobin as detected by benzidine staining. These Friend cell-erythroblast hybrids behave quite differently from the Friend cell-fibroblast cross. They retain only four human chromosomes but continue to synthesise haemoglobin provided Me₂SO is added to the culture medium. However, although both parent cells are haemoglobin producers, only mouse globin mRNA is detected in the hybrid cells, suggesting that only mouse haemoglobin is being made.

The authors have explained how they think these hybrid cell lines might be used for gene mapping. They propose that in the Friend cell-fibroblast cross the extinction of the mouse globin genes is controlled by a regulatory locus on the human chromosomes. If further chromosome losses were to occur then this locus might be lost, the globin genes re-expressed, and the chromosome(s) involved in controlling globin gene expression defined. Re-expression of originally suppressed genes has been observed in other cell hybrids so that their scheme might be feasible.

The human globin structural genes are probably located on the chromosome normally lost when a Friend cell-erythroblast hybrid is formed since they are not expressed in these hybrids. To assign the genes to chromosomes more accurately, hybrids will have to be isolated which contain only a few more chromosomes than normal Friend cell-erythroblast hybrids but which do synthesise human haemoglobin. If this is accomplished it will be interesting to compare the gene locations with those obtained by hybridising labelled globin mRNA to chromosomes *in situ*, a method already used to obtain tentative assignments of globin structural genes (Price, Conover, and Hirschhorn, *Nature*, **237**, 340; 1972).

they show 'dependence' in the sense that when the opiate is withdrawn from the cultures cyclic AMP levels rise to abnormally high values. Recovery from the 'addicted' state is slow, as it

requires the return of adenylate cyclase activity to normal values. These results support the suggestions made several years ago that opiates may act as enzyme inducers in the process of

addiction (Goldstein and Goldstein, *Biochem. Pharmacol.*, **8**, 48; 1963; Shuster, *Nature*, **189**, 314; 1961). They also agree with the suggestion by Collier, Francis, McDonald-Gibson, Roy and Saeed (*Life Sciences*, in the press) that abnormal formation of neuronal cyclic AMP in brain plays a key part in morphine addiction, and that some of the features of this state can be mimicked by administration of cyclic AMP or phosphodiesterase inhibitors in order to elevate brain cyclic AMP levels.

Another finding that may offer a crucial key to the understanding of the biochemical mechanisms underlying opiate drug actions is the discovery that chemical substances with morphine-like properties occur normally in brain. Hughes (*Brain Res.*, **88**, 295; 1975) recently described the isolation and characterisation of a low molecular weight peptide from bovine brain that is able to mimic the actions of morphine in pharmacological test systems. The new substance, called 'enkephalin', may be a neurotransmitter or modulator substance made and secreted normally in those brain regions that are rich in opiate receptors, and work on its further purification and identification is being actively pursued in several laboratories.

Although research in the area of opiate drugs is progressing at a bewilderingly rapid pace, it is clear that very important new facts are emerging in this hitherto enigmatic field. The combination of opiates, prostaglandins, brain peptides and cyclic nucleotides in a single branch of research is guaranteed to ensure that it will remain a glamour area for some time to come.

New support for autoimmune basis of myasthenia gravis

from Angela Vincent

The fifth conference on myasthenia gravis was held in New York on May 30-31.

MYASTHENIA GRAVIS (MG) is a disorder of neuromuscular transmission which is relatively rare but can be extremely disabling. It is characterised by weakness and increased fatigability of skeletal muscle which can be improved by acetylcholinesterase inhibition. Myasthenia is associated with thymic hyperplasia (75%) and thymoma (15%). Antibodies to skeletal muscle and thymic myoid cells have been demonstrated in about 30% of patients, and thymectomy often induces a remission. These features of the disease and its association with other immune disorders led Simpson

(*Scot. Med. J.*, **5**, 419; 1960) to propose that an autoimmune mechanism might be responsible, but attempts to implicate a serum factor blocking transmission have been largely unsuccessful.

Electrophysiological studies on biopsied intercostal muscle from MG patients by Elmqvist *et al.* (*J. Physiol., Lond.*, **174**, 417; 1964) showed reduced amplitude of miniature endplate potentials (m.e.p.ps) and it was suggested that there might be a reduction in the size of individual acetylcholine quanta released from the presynaptic nerve terminal. The possibility remained, however, that there might be reduced sensitivity of the postsynaptic membrane to released acetylcholine. This seems possible, since the total number of acetylcholine receptors has now been shown to be reduced in endplates from MG patients (Fambrough *et al.*, *Science*, **182**, 293; 1973; Green *et al.*, *Phil. Trans. R. Soc.*, **B270**, 551; 1975). However, there are morphological changes in MG endplates (Engel and Santa, *Ann. New York Acad. Sci.*, **183**, 46; 1971) and there is, as yet, no evidence as to whether this reduction in total numbers of receptors represents a reduction in density of functional receptors per unit area.

The first day of the meeting was concerned with neuromuscular transmission in MG and in a new animal model. In the last few years, many groups have purified the acetylcholine receptor (AChR) from the electroplax of electric eel and *Torpedo*. α -Bungarotoxin (α -Bgt), a snake toxin which blocks neuromuscular transmission irreversibly, has been used to assay the AChR in solution and in skeletal muscle. Antibodies raised in rabbits against electric eel AChR cross react with the animals' own AChRs *in situ* and produce progressive weakness, similar in many respects to that found in MG (Patrick and Lindstrom, *Science*, **180**, 871; 1973). Several speakers presented results from rabbits immunised against AChR from eel and *Torpedo*. A few days after the second injection, the animals developed weakness, usually progressing to death. Anticholinesterase treatment temporarily improved their condition. A decremental response to repetitive nerve stimulation could be demonstrated and m.e.p.p. amplitude was reduced. Circulating antibodies against electroplax AChR could be shown by *in vitro* techniques and by their ability to block carbachol-induced depolarisation in the intact electroplax.

J. M. Lindstrom (Salk Institute, La Jolla) described immunisation of rats with a single intradermal dose of eel AChR in adjuvants. About 8 days later they developed an acute episode of paralysis from which most recovered



A hundred years ago

IN NATURE, vol. xii, p. 98, you notice a paper by Dr. Hann on two rival theories of cyclones. According to one, "whirlwinds are formed mechanically by different streams of air meeting, and centrifugal force causes the central depression. The more modern theory regards a local depression as the first condition, causing an indraft resulting in a whirlwind through the earth's rotation: the primary depression is held to follow condensation of vapour."

The question is how the cyclone begins: whether the first depression is due to the centrifugal force of an eddy, or to the expansion of air in the upper strata from the heat liberated in the condensation of vapour. There need not be any controversy as to the dynamics of the cyclone after it is formed.

from *Nature*, **12**, 187; July 8, 1875.

in a few days. This was associated with severe focal degeneration of the muscle endplate and inflammatory infiltration of the muscles (A. G. Engel, Mayo Clinic, Rochester). V. A. Lennon (Salk Institute) showed that this was probably due to cell-mediated hypersensitivity: it could be abolished by prior thymectomy and could be transferred by T (thymus-derived) lymphocytes into X-irradiated recipients. A second chronic stage was also encountered in these animals. It began at about 30 days and was preceded by a rise in serum antibodies against the AChR. Morphologically the endplates had undergone repair, but the new junctions were simpler than normal and resembled those found in MG. There was evidence of neuromuscular block, reversed by anti-cholinesterase, but these animals did not show such severe weakness as the rabbits. The m.e.p.p. amplitude was reduced, however (E. M. Lambert, Mayo Medical School, Rochester), and the difference in clinical findings between rats and rabbits was thought to be due to the larger number of acetylcholine quanta released per impulse from rat terminals.

Is there a circulating antibody to the AChR in myasthenia gravis?

A. Bender (National Institute of Neurological Disease, Bethesda) showed that sera from patients with MG could inhibit an indirect labelling technique for AChR in cross sections of normal human muscle. S. Appel (Duke University Medical Center, Durham, North Carolina) and J. Lindstrom were able to detect binding of immunoglobulins from MG sera to Triton-solubilised receptors from normal human muscle;

but this binding did not appear to involve the acetylcholine recognition site of the receptor. Nevertheless, D. B. Drachman (Johns Hopkins University, Baltimore) had induced electrophysiological evidence of MG in immunosuppressed mice by repeated injections of MG globulins over a period of 10 days.

On the other hand, J. Keesey (University of California, Los Angeles) was unable to demonstrate any inhibitory effect of MG serum on α -Bgt binding to mouse diaphragm; E. X. Albuquerque (University of Maryland, Baltimore) had found no reduction in acetylcholine sensitivity in rat or human endplates after treatment with serum from MG patients; and injection of serum globulins or lymphocytes into rats produced no electromyographical evidence of neuromuscular block (T. Namba, Maimonides Medical Center, Brooklyn). Were these negative results due to immunoglobulins being unable to penetrate the intact neuromuscular junction? B. Fulpius (University of Geneva), using α -Bgt-labelled muscles and radioactively-labelled anti- α -Bgt *in vivo* and *in vitro*, clearly showed that the antibody was able to penetrate and bind to the motor endplate.

The second day was concerned with genetic, immunological and clinical aspects of MG. Histocompatibility antigens HL-A₂ and HL-A₈ were shown to be more prevalent in MG patients than in the normal population and divided the patients into two main groups: HL-A₂ being more common in older patients with thymomas and circulating antibodies to skeletal muscle, and HL-A₈ being associated with young females with thymic hyperplasia and no circulating antibodies (M. J. Oosterhuis, Wilhelmina Gasthuis, Amsterdam). The relative proportion and absolute numbers of T and B lymphocytes in MG were reported by several speakers, but no consistent pattern emerged from most of these studies. G. Birnbaum, however (Cornell University Medical College, New York), found decreased T-cell function in young, hyperplastic patients, and a significant rise in T cells and T-cell function after thymectomy. Further immunological studies may well be easier to relate to the disease process if tissue typing is included.

The effect of immunosuppressant therapy was discussed and various regimens for steroid treatment suggested. One interesting aspect presented by G. Matell (Karolinska Institute, Stockholm) was drainage of the thoracic duct undertaken in severe cases. These patients improved remarkably after 24–48 hours of drainage, although their condition deteriorated within 1–2 weeks of stopping the treat-

ment. In contrast, injection of the cell-free lymph back into the same patients produced deterioration within 30–60 minutes.

The animal models presented at this meeting showed considerable similarity to MG. Circulating antibodies raised against solubilised AChR can bind to, and directly block the receptor *in situ*; cell-mediated hypersensitivity to the receptor can produce symptoms of an acute nature, and it may be a necessary prelude to the humoral response in rats. Whether there is a circulating antibody in MG directed against the acetylcholine recognition site of the AChR, and capable of directly blocking the receptor, is still doubtful. It is possible, however, that the immunoglobulins that bind to solubilised human receptors, as shown independently by Appel and Lindstrom, have an indirect inhibitory effect on neuromuscular transmission *in situ*.

Denervation and re-innervation

from Shin-Ho Chung

BEFORE a foetal muscle is innervated, the fibre is 'supersensitive' to the chemical transmitter acetylcholine over its entire length. When a motor nerve forms an excitable junction with the muscle fibre, the chemosensitive area shrinks progressively and becomes restricted to the junction and its immediate surroundings (Diamond and Miledi, *J. Physiol., Lond.*, **162**, 393; 1962). A similar process occurs in adult muscle fibres following regeneration after destruction of the nerve (Miledi, *J. Physiol., Lond.*, **151**, 1, 1960; Axelsson and Thesleff, *J. Physiol., Lond.*, **147**, 178; 1959). Interest in this system stems partly from the fact that, during their supersensitive period, muscle fibres accept innervation from foreign

Pollution from plants

from Peter D. Moore

It is both well known and a source of considerable concern that the particulate matter of the atmosphere contains a burden of toxic heavy metals (see *Nature*, **247**, 510; 1974). In south-west England attempts have been made to monitor the fallout of heavy metals by analysing their levels in superficial leaf washings and in cuticular layers on vegetation (see, for example, Little, *Environ. Pollut.*, **5**, 173; 1973). The reverse situation is described by Beauford, Barber and Barringer in this issue of *Nature* (page 35); they have shown that heavy metals from the soil are absorbed by plant roots, are translocated and eventually released into the atmosphere in particulate form, perhaps with fragments of cuticular waxes. It has been realised for several years that plants can generate atmospheric particulate matter, but this is the first clear demonstration, using radioactive tracer techniques, that the movement of heavy metals from soil to atmosphere may accompany this process.

This being so, it may be necessary to reconsider the use of vegetation as a convenient filter by which heavy metal fallout from the atmosphere can be monitored. It is not usually known what proportion of cuticular and epicuticular metal is derived from the soil through the plant. In many situations the soil levels of heavy metals also reflect atmospheric fallout, in which case the leaf washings will still provide a useful index, but this is not so

where metals in soils are derived from other sources.

But perhaps the most important implication of this work, as the authors point out, is that industrial activity is not the sole source of atmospheric heavy metals. The question which must now be asked concerns the respective importance of these two sources. Here an answer may be found in palaeoecological work by Lee and Tallis (*Nature*, **245**, 216; 1973), who analysed the lead contents of peat samples taken from depths down to 2 m at an upland site in Derbyshire. Lead contents of almost 600 p.p.m. were found in the upper 20 cm of peat, but these fall to about 40 p.p.m. at depths below 20 cm (dated by radiocarbon determination at pre 1460 AD). At depths below 125 cm, the leaf level falls yet further to a fairly steady value of about 10 p.p.m. These samples predate the Roman conquest, during which there was some lead mining and smelting, hence it must reflect the background level of atmospheric lead, to which the vegetation is presumably making its contribution. Thus, even if this background were entirely due to the production of metal-containing particulate matter from plants, the current industrial and domestic production of lead still exceeds it by sixty times. In the midlands of England, therefore, atmospheric lead production by vegetation must be regarded a very minor contribution to the atmospheric content of heavy metals when compared with human sources.

nerves, with which they normally do not connect (Fex *et al.*, *J. Physiol., Lond.*, **184**, 872; 1966). These phenomena pose several salient questions. For example, what causes the spread of chemoreceptors after denervation? Is the development of high sensitivity to a transmitter substance a prerequisite for the establishment of a synaptic connection? What happens to 'incorrect' junctions when 'correct' nerves re-innervate muscle fibres? An article by Frank, Jansen, Lomo and Westgaard (*J. Physiol., Lond.*, **247**, 725-748; 1975) highlights these questions.

It was originally believed that a 'neural factor' released from nerve terminals prevented the spread of receptors beyond the synaptic region. Subsequent experiments showed this to be implausible. When nerve impulses flowing towards the muscle are blocked with an anaesthetic, while leaving the nerve otherwise undamaged, the muscle membrane becomes supersensitive, as it would if the nerve were cut. The development of supersensitivity can be prevented either by chronic nerve stimulation distal to the region of the block, or direct stimulation of the denervated muscle (Lomo and Rosenthal, *J. Physiol., Lond.*, **221**, 493, 1972; Frank *et al.*). These studies show that contractile activity of muscle fibres, innervated or denervated, inhibits the formation of extra-junctional receptors.

Because connections are formed only when muscle fibres are supersensitive to acetylcholine, it is tempting to postulate that the receptors are the membrane component which directly controls synapse formation. A pointer in this direction is the fact that the formation of contacts between denervated muscle fibres and foreign nerves can be arrested by maintaining the muscle activity by direct stimulation (Jansen *et al.*, *Science*, **181**, 559, 1973; Frank *et al.*). However, a recent demonstration (Van Essen and Jansen, *Acta physiol. scand.*, **91**, 557, 1974) that re-innervation proceeds normally in the presence of α -bungarotoxin, which binds irreversibly to acetylcholine receptors, makes such a generalisation premature.

There is conflicting evidence on the fate of 'incorrect' junctions when 'correct' junctions are re-formed. An earlier preliminary report (Cass *et al.*, *Nature*, **243**, 201, 1973; *Nature*, **243**, 185; 1973) claimed that 'correct' fibres render ineffective 'incorrect' synapses in axolotl muscles, while leaving them structurally intact. Such a suppression of 'incorrect' junctions is known to occur in mammalian skeletal muscles during development (Redfern, *J. Physiol., Lond.*, **209**, 701; 1970; Bagust *et al.*, *J. Physiol., Lond.*, **229**, 241; 1973). Frank *et al.* now show that both

foreign and original synapses can co-exist functionally for prolonged periods of time in adult mammalian muscles. Thus the ability to suppress inappropriate synapses is apparently lost in mature skeletal muscle.

The underlying cellular mechanism for afferent-efferent interaction, as illustrated by the denervated neuromuscular system, remains one of the mysteries in neurobiology. During the process of synaptogenesis in the nervous system, a target cell becomes receptive, selects an appropriate input, and suppresses the inappropriate ones. It is only through a combined immunological, biochemical and neurophysiological study of a simple system, such as the innervation of developing or denervated muscle, that we will gain insight into the rules governing the formation of specific nerve connections.

Hole transport in silica

from Andrew Holmes-Siedle

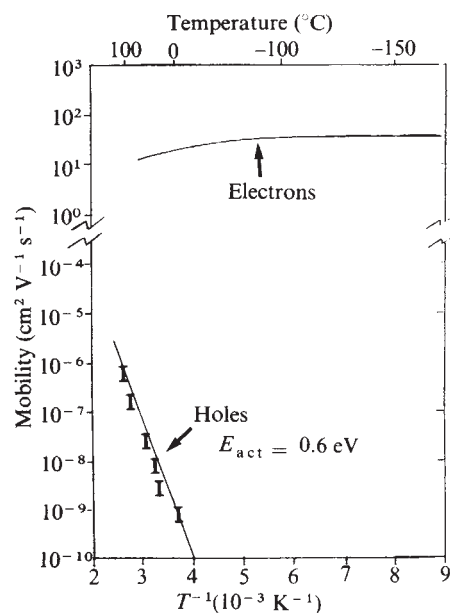
SILICA (SiO_2) is a very important material in solid state physics, electronics and optics. Although the crystal structure of quartz is complex and several inconvenient phase transitions of the lattice occur, the material is, on the whole, very well characterised. The amorphous form, fused silicon, presents a useful example of the simplest form of disorder, the only deviation from order being some variation in the Si-O-Si bond angles. In optics, the near-zero expansion and high ultraviolet transmission are extremely useful and, in electronics, the crystalline form is used in piezoelectric devices and the amorphous thin-film form is an essential part of all current forms of metal-oxide-semiconductor (MOS) device. Of course, the fact that quartz and silicates are the commonest minerals also help to make silica a technologically and economically important material.

The band-gap of SiO_2 is of the order of 9 eV and thus the electronic transport properties are not easily studied. But, as I discussed previously (*Nature*, **246**, 190; 1973), studies of the motion of electrons and holes in this material are now coming on apace. One of the motives for this work is the need to understand carrier transport in MOS systems and another is the general need to operate insulators under extremes of electric field or photon excitation. In the earlier article, the work of Hughes on electron transport in quartz and fused silica slabs—measuring photoconductivity after nanosecond pulses of X rays—was described. At that time, Hughes's results confirmed the general feeling that all cur-

rent was carried by electrons and that holes were virtually immobile, possibly being lattice-trapped in non-bonding oxygen orbitals (see, for example, Di Stefano and Eastman, *Phys. Rev. Lett.*, **27**, 1560; 1971). Some further measurements by Hughes (*Appl. Phys. Lett.*, **26**, 436; 1975) have now demonstrated a small contribution of the holes to photocurrents. The fact of the smallness is of great interest and will help towards clearing up controversy in the important field of carrier trapping in some insulators.

Hughes's measurements are summarised in the figure, which shows that, whereas electrons travel rather easily through the SiO_2 lattice, suffering only longitudinal optical phonon scattering, holes can essentially be 'frozen' at low temperature but still possess a measurable mobility at room temperature. This explains a hitherto puzzling result of Powell and Derbenwick (*IEEE Trans. nucl. Sci.*, **NS18**, no. 6, 99; 1971) who irradiated a 200 nm SiO_2 film with 10.2 eV light. Although the light was largely absorbed within a few nanometres of the surface, a positive charge sheet was found at the unilluminated side of the film, suggesting a finite mobility of holes or ions at room temperature. Arguments that ions were carrying charge are now unnecessary and, in general, the important work of distinguishing ionic from electron-hole transport effects in thin films, especially in MOS devices, can perhaps now proceed in a more orderly fashion.

The use of pulsed X-ray-induced photoconduction may become a useful general solid-state tool and will clearly



Mobility of holes and electrons in amorphous silicon dioxide films against reciprocal temperature, demonstrating differences in transport mechanism.

play a part in the important general question of charge motion and storage in wide-band solids.

Planetary collisions

from David W. Hughes

AT the dawn of the Solar System collisions between planets could have been so frequent and devastating that we now see only the scattered remnants of this scrummage. Interaction between planets in their present orbits is minimal, a situation which slowly built up as the Solar System aged. But small bodies, such as comets and asteroids, often have orbits which cross those of the planets and thus are intrinsically unstable. They may be eliminated by collision with a planet—either accreting to the planetary surface or (more rarely) being fragmented when they graze the planet's upper atmosphere—or they may be perturbed—either into shorter period orbits with aphelia near the planet's orbit (like the Jovian family of comets) or into hyperbolic orbits which take them out of the Solar System (like the space probes Pioneer 10 and 11).

Probabilities of encounter were first calculated in a classic series of papers by Öpik. Two fundamental approaches can be made to the problem, the analytical one which considers individual particles and their orbital elements and the numerical integration approach which uses random walk and Monte Carlo techniques applied to a large number of particles. These two approaches have been combined by Weidenschilling in a recent paper in *The Astronomical Journal* (80, 145; 1975). The probability of encounter between two objects with intersecting orbits is found to be simply a function of their relative velocities and one angular variable. These encounters will eventually occur even between particles with crossing orbits (orbits with the same range of heliocentric distances) because secular perturbations cause nodal precession and advancement of the perihelion, which finally results in intersections and then encounters.

Öpik (*Adv. Astron. Astrophys.*, 2, 219; 1963) found that the mean lifetime of the Apollo group of asteroids (which have orbits crossing those of Earth and Mars) is 10^8 years. Collisions would have caused their numbers to decay exponentially with time. So if these asteroids are remnants of an original group formed 4,500 Myr ago at the birth of the Solar System, there would have been 2.5×10^{19} of them at $t=0$, equivalent to a mass one hundred times that of the Sun. Clearly this is most unlikely, and thus the asteroids in this group cannot be permanent

members of the Solar System but must be continually replenished from the asteroidal belts and comets.

Weidenschilling comments on the differences in the encounter probability curves of the terrestrial and Jovian planets. This is partly explained by the great mass of Jupiter making it more likely than the inner planets to encounter material. But the mass of the planet also affects the ultimate fate of the small bodies encountered. The terrestrial planets, with orbital velocities several times greater than their escape velocities, have effective cross sections such that the ejection probability is only an order of magnitude higher than the collision probability. For Jupiter the orbital velocity is less than the escape velocity, the collision cross section varies with Joviocentric velocity and about 1,000 more particles will be ejected from the Solar System by Jupiter than collide with it.

Weidenschilling's figure for the probability of ejection by Jupiter is an

order of magnitude greater than Öpik's whereas his results for the terrestrial planets agree with those of Öpik. If Weidenschilling's Jupiter result is correct, the time scale for the dynamical elimination of comets must be two to four orders of magnitude greater than the time scale for disintegration. Thus the fact that thousands of dead cometary nuclei are not observed indicates that most comets disintegrate completely or that the dead nuclei are too small to be seen.

The equations given in Weidenschilling's paper also enable the efficiency of cometary ejection processes to be calculated. The author promises to apply these to the production of the Ört cloud of distant comets in a later paper. He also speculates that the total mass and angular momentum lost from the Solar System by cometary ejection might be of cosmogonical significance, indicating that the Solar System's angular momentum-mass balance might have varied with time. □

Type B hepatitis a kissing disease?

from Arie J. Zuckerman

THOUGH the importance of the parenteral and inapparent parenteral route of transmission of hepatitis B infection has long been recognised, it has become difficult to incriminate parenteral transmission in all cases of infection with hepatitis B virus (*Tech. Rep. Ser. No. 570*; World Health Organisation, 1975). Interest in various mechanisms of spread of this infection has flourished in recent years following the experimental demonstration of infectivity of serum by the oral route and epidemiological studies implying the possibility of venereal transmission of hepatitis B (*Nature*, 246, 59; 1973). Although the reported detection of hepatitis B surface antigen in semen and menstrual blood obtained from individuals with antigenaemia offers possible explanations for infection by the sexual route, the matter is far from settled.

The presence of hepatitis B surface antigen in a variety of body fluids and excretions such as urine, colostrum, milk, saliva, bile, faeces and sweat has not been consistently confirmed. In particular confirmation of the finding of the antigen in bile and faeces is still lacking in spite of intensive studies in various laboratories.

A recent report from Costa Rica has shed some light on possible non-parenteral routes of transmission of hepatitis B in endemic settings (Villarejos *et al.*, *New Engl. J. Med.*,

291, 1375; 1974). Serial samples of faeces, urine and saliva collected from chronic carriers of hepatitis B surface antigen and from patients with acute hepatitis B were tested by radioimmunoassay. The antigen was not found in any of 120 faecal extracts examined. The antigen was detected in three of 130 urine samples and all the positive samples contained occult blood. 274 saliva specimens from 93 chronic carriers were tested and the antigen was found in nearly 60% of specimens belonging to 75 subjects. It is interesting to note that the antigen was not found constantly in the saliva of the carriers, although all had circulating antigen at the time of sampling. Antigen was found in the saliva of 76% of 41 patients with acute hepatitis B, mainly during the first three weeks after the onset of clinical symptoms. The presence of the surface antigen in saliva both in carriers and in patients with the acute disease was not related to contamination with blood. These results show that salivary transmission may be a major mechanism of spread of infection in an area where hepatitis B occurs endemically. Villarejos and colleagues conclude therefore that transmission may occur directly by mouth-to-mouth kissing and in children by exchange of chewed toys and sweets. This presupposes of course that the antigen found in saliva is actually infective.

Prostaglandins and their intermediates

from a Correspondent

An international conference on prostaglandins was held in Florence on May 26–30.

ARGUABLY the most exciting findings of the whole meeting were revealed by S. Samuelsson (Karolinska Institute, Stockholm) in an introductory address. Recently Samuelsson's group found (Hamberg and Samuelsson, *Biochem. Biophys. Res. Commun.*, **61**, 942; 1974 and *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3400; 1974) that PGG_2 and PGH_2 , the endoperoxide precursors of prostaglandins (see *Nature*, **253**, 88; 1975), can, in lungs and platelets, also be transformed into other (non-prostaglandin) products including a hemiacetal derivative 'PHD'. Now Samuelsson's group have identified a very labile intermediate in the conversion of PGG_2 to PHD. This new intermediate does not have a classical prostaglandin structure and has been named 'Thromboxane' (see figure), because it is a very potent platelet aggregating agent. Since it is the first member of a new series of compounds and contains two double bonds it was further designated 'Thromboxane A_2 ': its metabolite PHD now becomes 'Thromboxane B_2 '.

Thromboxane A_2 has at least two other interesting properties; its half-life in aqueous solutions is about 30 s, and it strongly contracts strips of rabbit aorta. As a product of the prostaglandin biosynthesis cascade its generation is of course blocked by aspirin. These properties make it very likely that Thromboxane A_2 is in fact 'RCS'—the mysterious rabbit aorta contracting substance (released from lungs during anaphylaxis), first described by Piper and Vane in 1969 (*Nature*, **223**, 29). The importance of the endoperoxide system in platelet aggregation was emphasised by the Karolinska group's discovery of a subject deficient in the cyclo-oxygenase system (which converts arachidonic acid to PGG_2). Platelets from this subject showed reduced sensitivity to the aggregating action of adrenaline and collagen, but were aggregated by PGG_2 . The idea that the endoperoxides themselves, or their non-prostaglandin end products, may be important in other systems was later reinforced by M. Hamberg *et al.* (Karolinska Institute) who found elevated levels of endoperoxide and Thromboxane metabolites in perfused guinea-pig lung during anaphylaxis; PGG_2 and H_2 also had much more marked bronchoconstrictor activity than $\text{PGF}_{2\alpha}$. Both pieces of evidence suggested that the endoperoxides (or

their non-prostaglandin metabolites) could be important in the anaphylactic reaction. J. Vane (Wellcome Laboratories) presented evidence that PGG_2 and PGH_2 potentiated the inflammation produced in the rat paw by stimuli such as Carrageenin.

The question of the cellular origin of the substrate required for prostaglandin biosynthesis received some attention, though not perhaps as much as it deserved. Phosphatidylinositol was definitely implicated as a source of arachidonate for prostaglandin biosynthesis in the pig thyroid undergoing TSH stimulation (B. Haye, S. Champion and C. Jaquemin; Reims-Cédex, France) and in platelets exposed to a variety of aggregating agents (Iacona and Schoene, US Dept. of Agriculture, Beltsville). Some data from Vane's group suggested that phosphatidylcholine was the source of the arachidonic acid liberated during trauma of spleen tissue, and demonstrated that inhibition of phospholipase A_2 could prevent this initial step in the generation of prostaglandins from occurring.

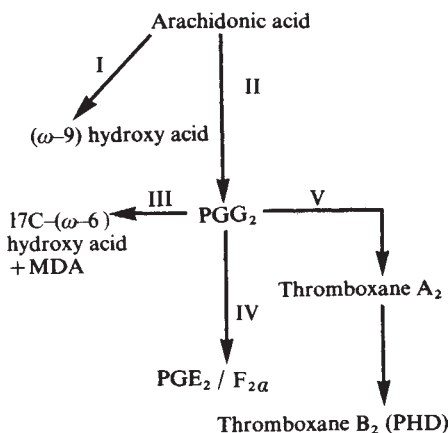
The section dealing with metabolism of prostaglandins aroused less interest: prostaglandin 15-hydroxy-dehydrogenase (PGDH) has been successfully extracted and purified from a variety of sources allowing good kinetic data to be obtained. One particularly ingenious and successful method of purifying the enzyme—by affinity chromatography using prostaglandins

as the ligand—was presented by E. Oliw, I. Lunden and E. Ånggård (Karolinska Institute). Throughout the conference, however, many workers were disappointed that so little attention was paid to the question of inhibiting this important enzyme: a really effective inhibitory drug would complement the use of the aspirin-like agents (inhibitors of prostaglandin biosynthesis) as a pharmacological tool to investigate the function of prostaglandins in the body. R. Flower *et al.* (Wellcome Laboratories) presented evidence that PGDH was a short-lived enzyme within the cell, the tissue levels falling very quickly after the administration of protein synthesis inhibitors.

Details of two important radioimmunoassay systems were presented at the meeting: F. Dray, B. Charbonnel and J. Maclof (Pasteur Institute, Paris) have devised techniques which eliminate most of the artefacts associated with the measurement of E and F-type prostaglandins in plasma and have thus reduced the falsely high readings obtained by many early workers. Their plasma levels for E-type prostaglandins of $<5 \text{ pg ml}^{-1}$ and for the F-type of $\leq 12 \text{ pg ml}^{-1}$ are probably the lowest recorded. Because prostaglandins are metabolised so rapidly, it is often more desirable to measure the major plasma (13, 14 dihydro-15 keto) or urinary (tetranor dioic acid) metabolites of prostaglandins. E. Granström (Karolinska Institute) presented some beautiful data on immunoassay systems designed for these purposes. The levels of metabolite were found to fluctuate throughout the oestrous cycle and were depressed during treatment with aspirin-like drugs. Confidence in the validity of both these assay systems was increased by the demonstration that the blood levels found corresponded well with those predicted from daily prostaglandin production rates.

Two clinical syndromes associated with the prostaglandin system were described: the platelet cyclo-oxygenase deficiency reported by Samuelsson's group (see above), and 'hyperprostaglandinaemia'—a syndrome probably due to a deficiency of PGDH—reported by A. Labrum, M. Lipkin and F. Dray (University of Rochester, New York and the Pasteur Institute, Paris). This condition was associated with periodic headache, vomiting, abdominal pain, diarrhoea and pyrexia.

Because of the arrangement of the conference, it was not possible to attend every session, so only certain aspects of the meeting are reported here. But it was clear that the impression felt by many at the end of this exciting conference was that the physiological role of prostaglandins—and now of their intermediates too—was as elusive as ever.



Alternative pathways of arachidonic acid metabolism in platelets. I, Conversion by a lipoygenase of arachidonic acid to an ω -9 hydroperoxide which is subsequently reduced to the corresponding hydroxy acid. II, Conversion of arachidonic acid by a cyclo-oxygenase to PGG_2 . III, Conversion of PGG_2 to a 17C-(ω -6) hydroxy acid and malondialdehyde. IV, Conversion of PGG_2 to $\text{PGE}_2/\text{F}_{2\alpha}$. This pathway is of minor importance in platelets. V, Conversion of PGG_2 to Thromboxane A_2 and Thromboxane B_2 (PHD). PHD is a hemiacetal derivative of 8(1-hydroxy-3-oxopropyl)-9, 12L-dihydroxy-5, 10-heptadecadienoic acid.

articles

Buried Mesozoic volcanic–plutonic fronts of the north-western Pacific island arcs and their tectonic implications

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Detailed magnetic surveys around the islands of Japan have revealed the presence of Mesozoic volcanic and plutonic belts at depth beneath the continental shelf. These belts, which can be correlated with those on the mainland of eastern Asia, provide a record of the migration of island arcs since the opening of marginal seas during the Tertiary.

DETAILED surveys (refs 1, 2 and Geographical Survey Institute, Japan, unpublished) have revealed remarkable features in the total magnetic intensity anomalies around Japan and the surrounding continental shelf and magnetic anomalies from Russian data^{3,4}, and oceanic magnetic lineations compiled by Uyeda *et al.*⁵ have also provided evidence of some interesting features (Fig. 1).

Most significant is the discovery of a north–south, positive anomaly belt coupled with a narrower negative belt to the west, running from the eastern side of north-eastern Honshu to the southern part of Hokkaido (Fig. 1 i) with a maximum amplitude exceeding 500 nT. A similar positive anomaly belt runs from the south-east of Hokkaido to Kamchatka, along the Kuril Island Arc (Fig. 1 ii). These two magnetic lineations can be distinguished from lineations in the oceanic basins because their locations coincide with the continental crust. Furthermore, the trend intersects that of the oceanic lineations (Fig. 1 iv) in the western Pacific Basin up to the Japan Trench. Oceanic anomaly iv (Fig. 1) disappears in the middle of the continental shelf in the Japan Trench approximately at the location of the 3,000-m isobath. As the crust under the landward slope of the Japan Trench is not oceanic, the presence there of oceanic anomalies suggests that oceanic crust has been thrust beneath the island arc. By the same reasoning we believe that the positive magnetic lineation (Fig. 1 iii) which is observed at the landward slope of the Kuril Trench and which is roughly parallel to both the trench and the oceanic lineations, is of oceanic origin. Other positive magnetic lineations at ocean-to-continental boundaries occur at Primorye, Sikhote-Alin, Sakhalin and in the Okhotsk Sea (Fig. 1).

The correlations between the positions of the isolated, patch-shaped, positive (or negative) magnetic anomalies in the middle of Honshu and Hokkaido and the line of Quaternary volcanoes known as the Quaternary Volcanic Front (QVF)⁶ (Fig. 1) are remarkable. Approximately 30 km east of the QVF (Fig. 1) a Miocene volcanic front has been identified geologically but is not clearly reflected by the magnetic anomalies.

Identification of buried Mesozoic volcanic–plutonic belts

Figure 2 shows profiles of total magnetic intensity anomalies, and bathymetry and magnetisation models which can account for the magnetic anomalies that cross the two continental magnetic lineations (Fig. 1 i and ii). The magnetic anomalies along line A in Fig. 1 can be modelled by a two-dimensional magnetised block trending north–south, with an intensity of magnetisation 5×10^{-3} e.m.u. cm⁻³, a direction of magnetisation of N45°W and an inclination of 53°. The magnetised block is 20 km thick with its top 10 km below the ocean. The most important characteristic of this model is that its magnetisation is largely westerly, in contrast to the present magnetic field, which suggests lineation i (Fig. 1) is caused by a westerly remanent magnetisation. Even if the magnetic anomalies are affected by magnetisation induced by the present magnetic field, the westerly tendency of the remanent magnetisation is by no means altered; on the contrary, it would be further emphasised. The magnetic anomalies along line B (Fig. 1), on the other hand, can be explained by a two-dimensional block trending ENE to WSW, magnetised with an intensity of 5×10^{-3} e.m.u. cm⁻³ in the direction of the present field. Almost the same results can be expected from the other sections of the continental magnetic lineations i and ii.

The fact that lineation i may result from a westerly remanent magnetisation is consistent with the natural remanent magnetisation of the Lower Cretaceous granitic samples obtained by Kawai *et al.*⁷ from the Kitakami and the Abukuma massifs in north-eastern Honshu. They proposed an anticlockwise rotation of north-eastern Honshu and a clockwise rotation of south-western Honshu some time after the Lower Cretaceous. As the continental magnetic lineations exist in areas of continental crust, and as a rotation of Japan should accompany any rotation of continental crust, the estimated westerly remanent magnetisation could be further evidence of the anticlockwise rotation of north-eastern Honshu. The magnetisation of lineation ii, on the other hand, cannot necessarily be taken to indicate the absence of rotation with respect to the Hokkaido–Kuril–Kamchatka Arc, because of the nearly east–west geographical trend of the supposed magnetised block. Although it is uncertain whether or not the magnetisation of lineation ii deviated in direction from the present magnetic field, we assume that lineation ii results mainly from a remanent magnetisation with approximately the same direction as the present magnetic field, and that little or

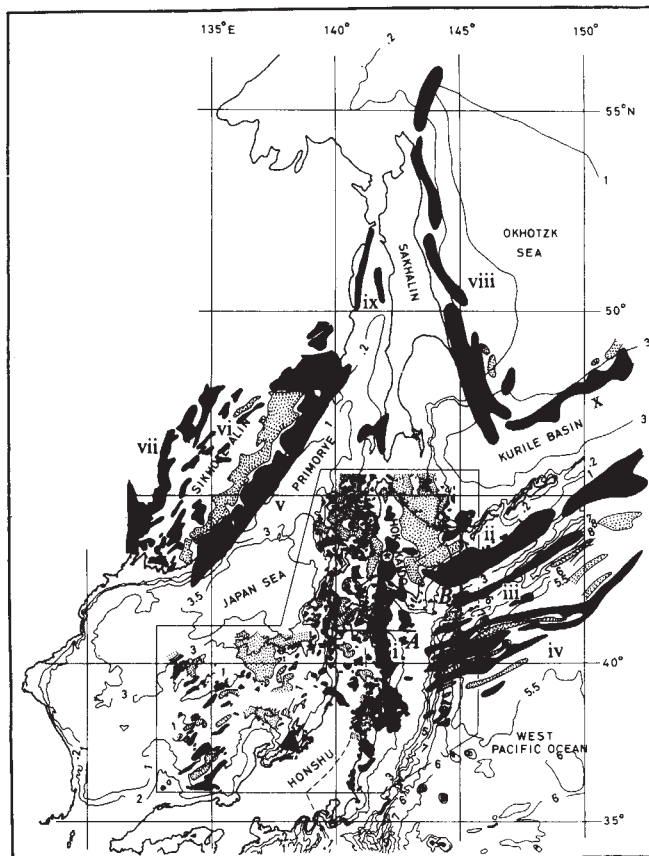


Fig. 1 Total magnetic intensity anomalies in and near Japan. The area enclosed by solid lines has been measured in detail in runs approximately 2 miles apart. Solid areas, positive anomalies; dotted areas, negative anomalies below -200 nT; blank areas, either anomalies between 0 and -200 nT, or no measurements. i-x, Marked lineated anomaly patterns; A and B, locations with magnetic profiles illustrated in Fig. 2; dashed curve, Quaternary Volcanic Front. Bathymetric contours are in metric units. Asterisk, site of a test drilling in the Sorachi region, Hokkaido.

no appreciable rotation has occurred with respect to the Hokkaido-Kuril Arc.

There are two lines of evidence from which the material which causes the continental magnetic lineations can be identified: a test drilling⁸ at Sorachi Province, Hokkaido, which coincides with the northern end of lineation i; and the result of measurements⁹ of magnetic susceptibilities and remanences of the drilling cores (Fig. 3). The drilling went down to a depth of 3,712.97 m, where the drilling bits hit a basement layer. The basal layer dips from west to east, and the

sedimentary layers show significant unconformities (Fig. 3). Analyses of gravity anomalies combined with geology and drilling results¹⁰ indicate that the basal layer consists of metamorphosed basic volcanics of Lower Cretaceous age, corresponding to the eastern slope of an anticline which aligns with lineation i. The magnetic susceptibilities of the drilling cores (Fig. 3) indicate that the metamorphosed basic volcanics of the basal layer are as strongly magnetised as the recent volcanics. These measurements have also shown that the magnetic Q ratio of the basement rocks is 1 or somewhat smaller. Although it is not certain whether the anticlinal basement is the origin of lineation i, we think it probable that either the anticlinal basement or a belt of ultramafic dyke material beneath it may be responsible for the anomaly. If that is so it can be inferred that volcanic-plutonic activity was initiated in Lower Cretaceous times or earlier, below the lineation. This volcanic-plutonic belt may be compared to the belts of Mesozoic volcanic-plutonic outcrops¹¹ on the Japan Sea side of south-western Honshu. As for lineation ii, no drilling data are available at present. But the areas are known to be abundant in Cretaceous volcanics, serpentines and other rock types¹², which seems to indicate the existence of Cretaceous igneous activity.

Comparison with other continental magnetic lineations

Magnetic anomalies in the Primorye and Sikhote-Alin provinces of eastern Asia show a distinct correlation to the belts of intrusions of intermediate to ultrabasic compositions¹³. The two eastern lineations (Fig. 1 v and vi) are located at both sides of the Sikhote-Alin granitic massif. One of the lineations, which runs along the coast of eastern Primorye and extends over the continental shelf on the Japan Sea side, is a coupled belt comprising a negative belt on the west and a positive belt on the east. Although the amplitudes of the magnetic anomalies are not reported in the Russian data, we judge, from the trend of the lineation (about $N40^\circ E$), that along the coast of eastern Primorye lineation v is caused by a normally magnetised block, though the absence of negative anomaly belts associated with lineations vi and vii, which have the same trend, is not easily explicable. The close correlation between the ultramafic intrusive belts of eastern Asia and the magnetic lineations could indicate that the magnetic anomalies east of north-eastern Honshu are also caused by ultramafic intrusions. The orogenesis of the Sikhote-Alin Massif is supposed to have occurred during the Upper Cretaceous. Although the ages of the intrusive rocks around its sides are not reported, we think it likely that the Sikhote-Alin orogenesis is intimately related to the neighbouring intrusions (which therefore also occurred in the Upper Cretaceous or later). The sources of the magnetic lineations at Sakhalin and along the northern margin of the Kuril Basin are not known. We

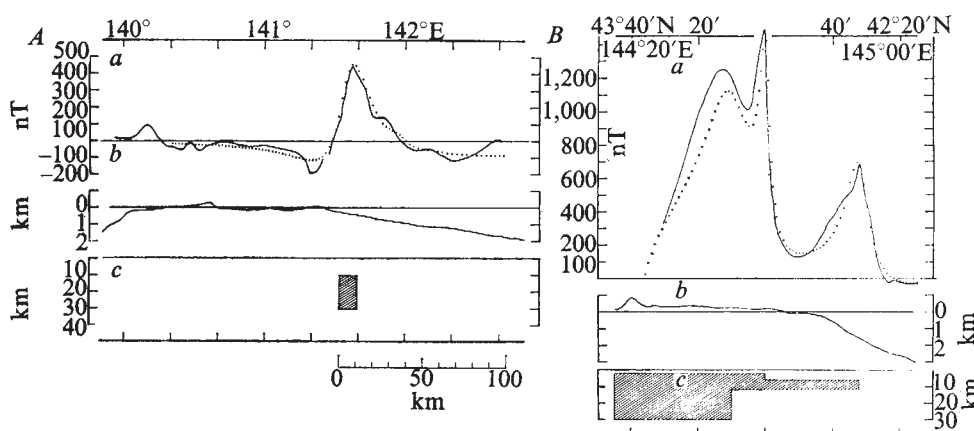


Fig. 2 Profiles of total magnetic intensity anomalies (a), bathymetries (b) and magnetisation models (c) across lineation i along line A and lineation ii along line B. The intensity of magnetisation is assumed to be 5×10^{-3} e.m.u. cm^{-3} for both cases. A: Present magnetic field—declination, $N8^\circ W$; inclination, 53° ; horizontal component, 28,500 nT. Magnetisation model—declination, $N45^\circ W$; inclination, 53° . B: Present magnetic field—declination, $N8^\circ W$; inclination, 56° ; horizontal component, 27,200 nT. Magnetisation model—declination, $N8^\circ W$; inclination, 56° .

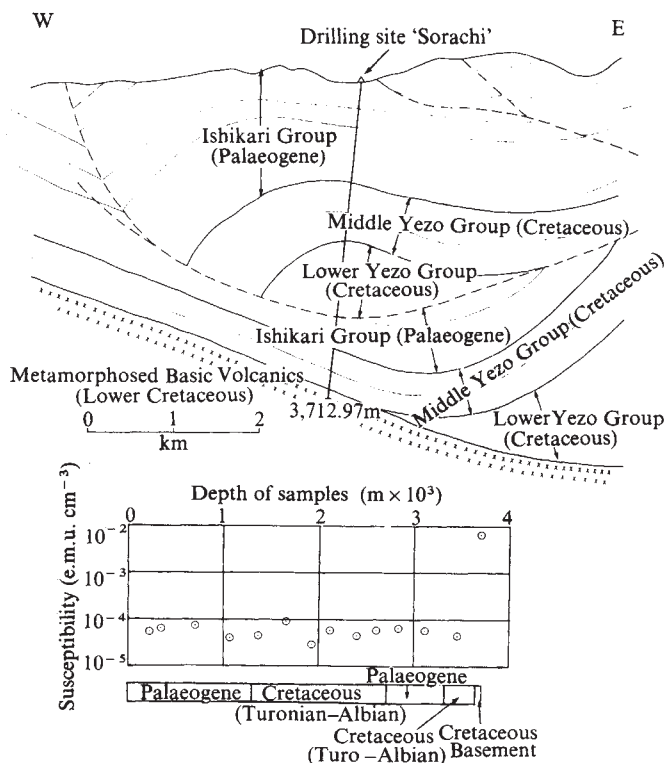


Fig. 3 Upper: east to west section of geological layers estimated from the result of a test drilling at the Sorachi region, Hokkaido. The drill penetrated to a depth of 3,712.97 m. Dashed lines, surfaces of unconformity of sedimentary layers. Crosses, basal layer. Lower: susceptibilities of the drilling cores at the Sorachi regions. Ordinate, susceptibilities (e.m.u. cm⁻³); abscissa, depths of the samples (m). Ages of the drilling cores shown at bottom.

which it takes for a buried dyke to cool from 1,000 °C down to 500 °C indicates that about 10 Myr are needed¹⁵. Such a long cooling time together with the slower accumulation of intrusive materials suggests that at least several million years of steady magnetic polarity would be necessary before a continental intrusion can become magnetised in a fixed direction. Therefore, only two possible ages—Jurassic and Cretaceous—can be assigned to the intrusive belts. Thus, we think that most of the magnetisation which produced lineations i and ii may have been acquired during the long Cretaceous normal epoch, presumably between the Aptian and the Albian. On the other hand, the intrusions found at Primorye, which look somewhat younger than those of Honshu, may have been magnetised during the Cenomanian–Turonian. It is interesting that if this hypothesis is correct, the smooth magnetic zones of the oceanic basins correspond to the most magnetically anomalous areas of the continents.

Inference of the movement of the island arcs

The openings of the Japan Sea and the Kuril Basin are inferred to have occurred during the Tertiary. As it is generally accepted that the formation of island arcs is the result of active volcanism caused by the descent of an oceanic plate¹⁶, a tracing of the history of the volcanism and/or the occurrence of the magnetic lineations could reveal the evolution of island arc formation. Unlike the oceanic magnetic lineations, the continental magnetic lineations are not necessarily aligned according to their ages. It seems probable, however, that the igneous activity responsible for the magnetic lineations occurred at closer spacings than are now recognised: for example, we suggest that the Lower Cretaceous intrusion east of north-eastern Honshu must have been closer to the outer margin of eastern Primorye, where another continental

assume, however, that they are all caused by basic or ultramafic intrusions.

Dating of the continental magnetic lineations can be attempted using the magnetic reversal time scale. The Mesozoic magnetic reversals¹⁴ include two epochs during which the magnetic polarity remained normal for a long time: the first occurred before the Kimmeridgian (148 Myr BP); the second during the Aptian–Comacian (111.5–84.5 Myr BP). As remanent magnetisation is produced only when magma has cooled sufficiently, a steady remanent magnetisation cannot be acquired by continental intrusives (which seem to accumulate much more slowly than oceanic intrusives) during rapid magnetic reversals. Moreover, an estimation of the period

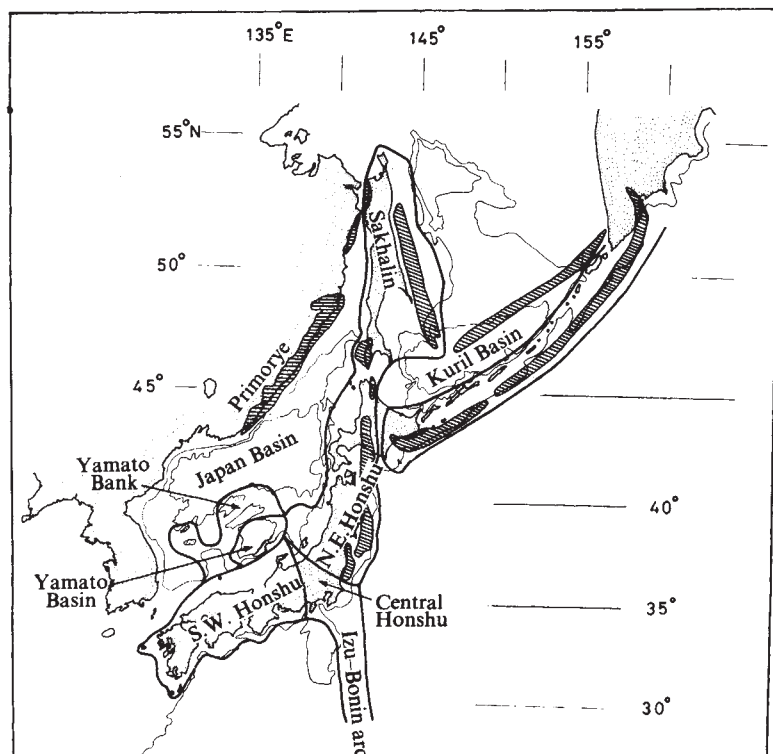


Fig. 4 Boundaries (thick solid lines) showing crustal blocks of Japan, Kuril and Sakhalin provinces. Hatched areas, marked magnetic lineations.

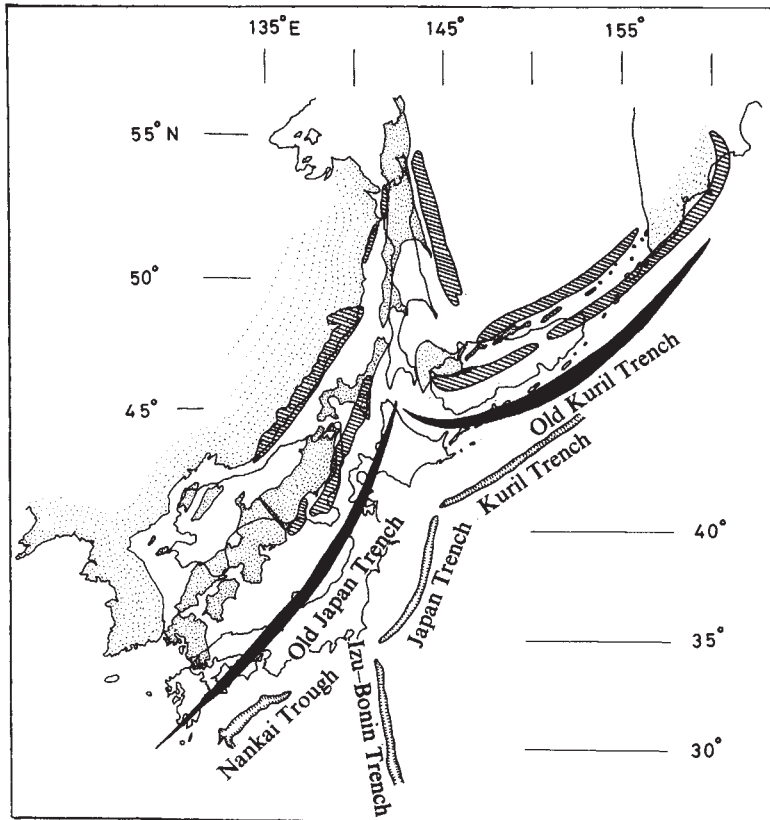


Fig. 5 Reconstruction of a possible feature of the Japan-Sakhalin-Kuril arcs before the Tertiary. Dotted areas, old continent and islands; hatched areas, marked magnetic lineations; Broad solid areas, possible locations of the old Japan and the Kuril trenches.

magnetic lineation is observed. The locations of the volcanic belts may be affected¹⁷ by both the location of the subduction zones and the dip of the descending plates, resulting in a disorderly configuration of the volcanic-orogenic belts with time. We think, however, that the distances between successive (Cordilleran-type) orogenic belts observed at the continental margins are, in most cases, 200–300 km. If this should hold for general cases, the distance of more than 800 km at present recognised between the massifs of north-eastern Honshu and the Sikhote-Alin suggests a more recent detachment of Honshu from the eastern Asiatic mainland; that is, a later opening of the Japan Sea. Although this argument requires no magnetic evidence, the documentation of magnetic lineations and their sources has enabled us to generalise the argument to apply to marine areas.

Figure 4 shows the tentative boundaries of continental blocks into which the island arcs can be divided and reconstructed so as to form the eastern Asiatic mainland at a time before the Tertiary. We have used our own criteria of reconstruction.

- We regard parts of an island arc with continental magnetic lineations as a single crustal block.
- Marginal basins such as the Japan Basin, the Yamato Basin and the Kuril Basin are replaced by continental crust.
- The Yamato Bank is regarded as continental crust.
- The central portion of Honshu together with the Izu-Bonin Ridge are excluded because they are very young.
- The Honshu, the western part of Hokkaido, and Sakhalin are treated as a single block because they seem to be connected across the Tsugaru and the Soya Straits, as is verified by seismic¹⁸ and gravity results¹⁹.
- The eastern part of Hokkaido is connected to the block of the Kuril Arc.
- Each block is either translated or rotated in such a way that the continental magnetic lineations become parallel and as close to each other as possible. In this case, north-eastern Honshu turns clockwise, whereas south-western Honshu turns anticlockwise.

Figure 5 shows a reconstruction for the Japan-Sakhalin-Kuril-Kamchatka region. A most significant corollary has been obtained as a result of dividing the Hokkaido district into western and eastern parts. The Hidaka Massif, trending north-south in the middle of Hokkaido, is known as a unique collision-type zone²⁰ showing a reversed, paired metamorphic belt²¹, and it may be accounted for in terms of the collision of two island arcs. The area west of the Hidaka Mountain, that is, the Ishikari zone, is one of the most highly fractured and folded zones in Japan. As seen from the results of the test drilling at the Sorachi region (Fig. 3), the eastern Palaeogene sedimentary layers step over the western portions of the same layers. This suggests that intense folding and fracturing occurred in and after the Palaeogene. Results from seismic refraction surveys (S. Asano *et al.*, unpublished), P-wave travel-time anomalies (T. Moriya *et al.*, unpublished) and electrical conductivity anomalies (Y. Nishida, unpublished) also indicate abrupt changes of crustal thicknesses and depths of conductive layers in the mantle between western and eastern Hokkaido. This evidence suggests that the Hidaka Massif was built when the eastern crust of Hokkaido rode over the western crust during a collision of two island arcs, when the Honshu-Hokkaido-Sakhalin block was drifting south-eastwards, away from eastern Asia, and the Kuril Arc was moving southwards. A convergence of crusts occurred at the present Hidaka Mountain area. The reversed paired metamorphism of the Hidaka region may be regarded as a result of the upheaval and exposure of the crust on the eastern side, which was produced by the igneous activity of the former Kuril subduction zone.

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Growth inhibition of animal cells by succinylated concanavalin A

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A non-agglutinating lectin preparation, succinyl-con A, inhibits the growth of untransformed 3T3 mouse fibroblasts in a manner that mimics density-dependent growth inhibition. The growth inhibitory interaction between succinyl-con A and cells occurs exclusively during the mitotic and/or early G₁ phase of the cell cycle.

A DIRECT role for the cell surface in the maintenance and regulation of cell growth has been suggested but never convincingly demonstrated. Lectins which bind sugars have been successfully used to examine the composition and properties of that part of the cell surface that is available to the extracellular environment. In a number of cell types a correlation has been shown between saturation density in tissue culture and both agglutinability with lectins¹ and, in defined conditions, tumorigenicity². Several investigations have demonstrated a correlation between the agglutinable surface configuration and growth of both normal and transformed cells³.

We have reported previously that normal cells during mitosis also exhibit the agglutinable surface configuration and have suggested that this mitotic surface change might be involved in growth regulation⁴. In this report we present evidence that a non-toxic, non-agglutinating lectin preparation interacts with 3T3 mouse fibroblasts during the mitotic phase of the cell cycle and induces a "density-dependent like" growth inhibition.

Growth of 3T3 cells inhibited by succinyl-con A

The enzymatic preparation of non-agglutinating concanavalin A (con A) by protease^{5,6} has proved difficult (although a detailed preparative procedure has been described by Evans and Jones⁷) as compared with the succinylation procedure of Gunther *et al.*⁸. A slight modification of that procedure, that is, scaling up the preparation sixfold and introducing affinity chromatography on Sephadex G-50 after the final succinylation, has reduced the contamination by tetrameric con A from approximately 5% to less than 0.1% when assayed by sedimentation in the analytical ultracentrifuge and glycogen precipitation. There were no detectable differences in the growth inhibitory properties of these various succinyl-con A preparations.

Succinyl-con A inhibits the growth of untransformed 3T3 cells in a way that depends on concentration (Fig. 1a). The growth inhibition seems to be specific for succinyl-con A since as much as 500 µg ml⁻¹ succinylated bovine serum albumin (succinyl-BSA) has no inhibitory effect on cell growth and, furthermore, since growth inhibition by 500 µg ml⁻¹ succinyl-con A can be prevented (95% that of control) by

10⁻² M α-methyl-D-mannoside, a hapten specific for con A. Succinyl-con A inhibited cells accumulate in the G₁ phase of the cell cycle (determined by impulse cytophotometry) and show a rate of ³H-thymidine incorporation per cell identical to that of a density inhibited monolayer.

Other experiments suggest that succinyl-con A does not inhibit growth by binding to, and therefore "removing" from the medium a necessary growth factor, but rather that succinyl-con A interacts directly with the cell. First, Dulbecco's modified Eagle's medium preincubated with 500 µg ml⁻¹ succinyl-con A at 37 °C for 24 h, and then filtered by diaflow ultracentrifugation to remove the succinyl-con A before the addition of calf serum, supported growth to the same level as untreated medium. Second, an increase in the serum concentration of the growth medium results in an increase in the final density of both control and succinyl-con A-treated cells. Significantly, however, the percentage of growth inhibition at a given concentration of succinyl-con A is independent of the serum concentration.

Inhibition of growth by succinyl-con A seems to be non-toxic since it is reversible simply by removing the medium containing the succinyl-con A and replacing with fresh medium (Fig. 1b). Succinyl-con A-inhibited cells show the same percentage of cells responding to serum pulses with succinyl-con A as control cells show to serum pulses without succinyl-con A. The plating efficiency of cells inhibited by succinyl-con A is similar to that of cells from a density-inhibited monolayer.

It should be noted that although quantitative differences in the growth inhibitory capacity of succinyl-con A can vary as much as twofold when using different cell clones and different succinyl-con A preparations, the qualitative effects are consistent. The data presented here reflect some of our least active succinyl-con A preparations. Some preparations decrease saturation densities to 50% with 200 µg ml⁻¹ succinyl-con A.

Inhibition of growth is dependent on cell density

An interesting aspect of growth inhibition induced by succinyl-con A is that the cell density at which growth ceases is independent of the initial, that is, plating, density (Table 1). It seems that growth inhibition is some function of both succinyl-con A-cell interactions and cell-cell interactions and that succinyl-con A-cell interactions and cell-cell interactions are similar effects which in some way can substitute for one another to inhibit growth.

Succinyl-con A acts only during mitosis and/or early G₁

Synchronously growing 3T3 cells were tested for their susceptibility to succinyl-con A at various times during the

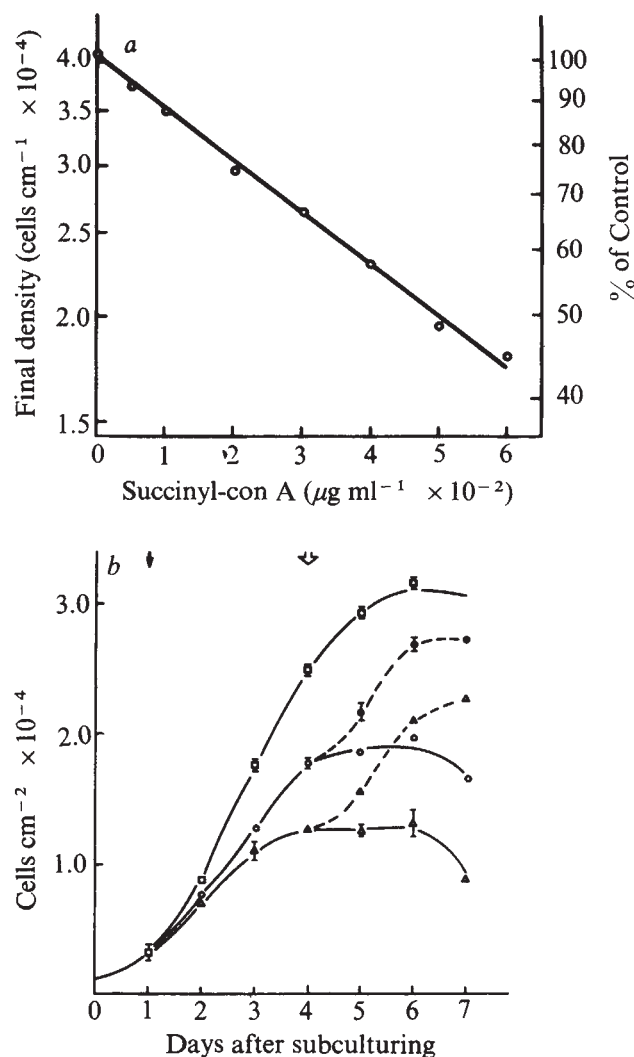


Fig. 1 Characteristics of succinyl-con A-induced growth inhibition of 3T3 mouse fibroblasts. 3T3 cells were subcultured into 3.5 cm tissue culture dishes containing Dulbecco's modified Eagle's medium (DME) + 10% calf serum; the medium was changed after 1 and 4 days of growth to DME + 5% calf serum $\pm$ succinyl-con A as indicated. Cell numbers were determined using a Coulter counter after removal with a buffered EDTA (0.54 M)-trypsin (0.05%) solution; each point represents duplicate samples counted twice. *a*, Concentration dependence of succinyl-con A-induced growth inhibition; *b*, reversibility of growth inhibition. $\square$, control; $\circ$, 250 $\mu\text{g ml}^{-1}$ succinyl-con A; $\triangle$, 500 $\mu\text{g ml}^{-1}$ succinyl-con A; $\bullet$ and $\blacktriangle$, succinyl-con A removed on d 4 and replaced by fresh medium containing 5% calf serum.

Table 1 Density dependence of succinyl-con A-induced growth inhibition

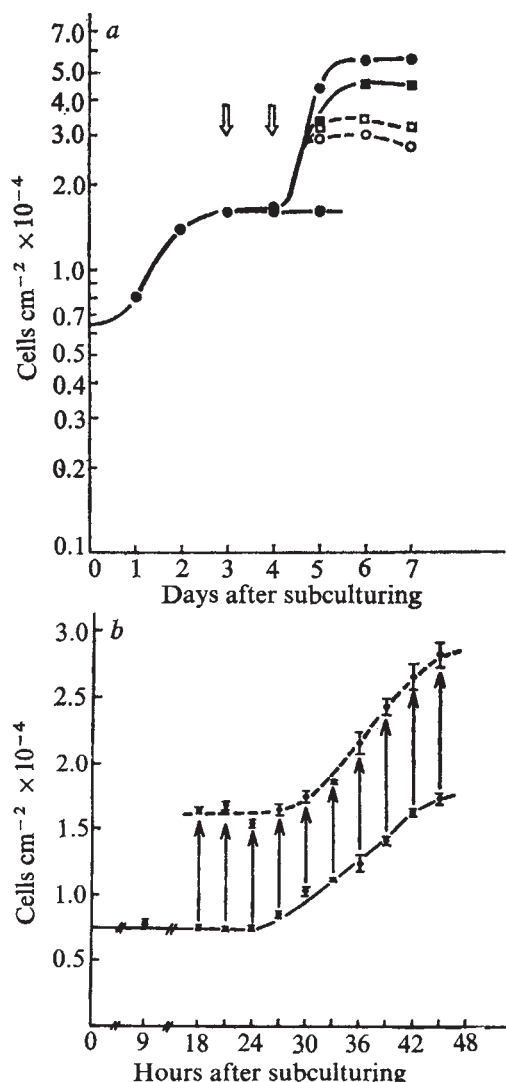
Initial density (cells cm ⁻² × 10 ⁻⁴)	Final density (cells cm ⁻² × 10 ⁻⁴)	% of control
<i>a</i> Controls (0.15–0.50)	2.80 $\pm$ 5%	
0.15	1.90	67.7
0.35	2.01	71.9
0.50	2.14	76.4
<i>b</i> Controls (0.18–0.51)	3.40 $\pm$ 5%	
0.18	2.27	66.8
0.35	2.44	71.8
0.51	2.45	72.1

Experimental conditions were as described in Fig. 1; *a*, Cells grown in DME+4% calf serum + 250 $\mu\text{g ml}^{-1}$ succinyl-con A; *b*, cells grown in DME + 5% calf serum + 250 $\mu\text{g ml}^{-1}$ succinyl-con A.

cell cycle (Fig. 2). Even though succinyl-con A may be present from the initiation of a new cell cycle in G₀, through the rest of G₁, S, and G₂, one round of cell division is not inhibited; the cells go through mitosis and come to rest in G₁ (Fig. 2*a*). Indeed, if succinyl-con A is removed just before mitosis, the cells continue to grow, going through a second round of the cell cycle essentially as if they had never been in the presence of succinyl-con A; succinyl-con A added immediately before the cells enter mitosis, however, causes a cessation of growth following the completion of that mitotic phase.

By adding succinyl-con A to a synchronously growing population of 3T3 cells at various times throughout the mitotic period, it was possible to obtain a more detailed understanding of the inhibition induced by succinyl-con A

Fig. 2 Cell cycle dependence of succinyl-con A-induced growth inhibition. *a*, 3T3 cells were subcultured into DME + 2% calf serum. When growth had become stationary³⁷, the medium was replaced with DME + 10% calf serum $\pm$ succinyl-con A (500 $\mu\text{g ml}^{-1}$) and replaced again 23 h later, just before mitosis: $\bullet$, control; $\blacksquare$, succinyl-con A at the first medium change, none at the second; $\square$, succinyl-con A at the second medium change; $\circ$, succinyl-con A at both medium changes. *b*, 3T3 cells from a 2-d-old confluent monolayer were subcultured into DME + 10% calf serum; succinyl-con A (final concentration 500 $\mu\text{g ml}^{-1}$) was added at various times during the mitotic period; cells at a density indicated by the continuous line (lower curve) at the time of addition of succinyl-con A grew to a corresponding final density indicated by the broken line (upper curve); each point represents three samples counted in duplicate; cell numbers were determined as described in Fig. 1.



(Fig. 2b). As would be predicted in these conditions if the sensitive period of the cell cycle is mitosis and/or nearly G_1 , the final density of a cell population is higher if more cells are allowed to pass through mitosis before the addition of succinyl-con A. The new information obtained from this experiment is that the midpoint of the "final density" curve (upper curve, Fig. 2b) is shifted to a time in the cell cycle that is approximately 1.5 h later than the midpoint of the "mitosis" curve (lower curve, Fig. 2b)—in three separate experiments, each done in triplicate, the shift was between 1.5 and 2 h. These data suggest that the phase of the cell cycle during which cells are sensitive to the inhibitory effects of succinyl-con A may be early in G_1 , a time during which previously rounded cells, which have just completed mitosis, are returning to a flat morphology but still retain the "agglutinable" cell surface configuration.

to inhibit the growth of transformed cells by interacting with the cell surface. Two preliminary experiments using succinyl-con A covalently linked to polyacrylamide beads inhibited the growth of 3T3 cells to at least 30%.

Trowbridge and Hilborn³² have reported that succinyl-con A binding to nearly confluent monolayers of 3T3 and SV3T3 cells is saturated at a concentration of $100 \mu\text{g ml}^{-1}$ at 0°C in phosphate buffered saline, and they concluded that this concentration has no appreciable effect on cell growth. A closer examination of their data, however, seems to indicate approximately a 30% inhibition of 3T3 cell growth at $100 \mu\text{g ml}^{-1}$ succinyl-con A, an inhibition which can be reversed by 50 mM α -methyl-D-mannoside. Our findings that concentrations of succinyl-con A higher than $100 \mu\text{g ml}^{-1}$ have a marked, specific and non-toxic effect on the growth of 3T3 cells, may not be contradictory to the binding studies of

Table 2 Summary of surface characteristics of interphase, mitotic and transformed cells

Surface features*	Interphase cells	Mitotic (and early G_1) cells	Transformed cells
Morphology—scanning electron microscope	Flat and relatively smooth surface	Round with many surface microvilli ¹⁸	Less flat with irregular surface features including microvilli, blebs and/or ruffles ⁹
Ease of detachment from substratum	Difficult	Easy	Easy
Electrophoretic mobility	Low	High ¹³	High ^{11,12}
Lectins			
(a) Agglutinability ¹⁴	(a) Low	(a) High	(a) High
(b) Binding of fluorescent labelled lectin ^{4,15}	(b) Low	(b) Higher	(b) Higher (only some)
Glycoproteins	Completely glycosylated	More fucose and sialic acid containing glycopeptides ¹⁷	Incompletely glycosylated. More fucose and sialic ¹⁶ acid containing glycopeptides
Glycolipids			
(a) Forssman antigen reactivity	(a) Low	(a) High ¹⁹	(a) High ¹⁸
(b) Gangliosides (for reviews see refs 3 and 23)	(b) Completely glycosylated or "higher" gangliosides	(b) Not determined	(b) Reduction of "higher" gangliosides—accumulation of precursors
Cell contact dependence ²⁰ of surface galactosyl-transferase activity	Dependent	Independent	Independent
Hynes protein	High levels	Very low levels ²²	Absent ²¹
Surface antigens (for reviews see refs 3 and 23)	Relatively few	New antigens appear—some disappear	New antigens appear—some disappear
Virus induced fusion	Low	High ²⁴	Not determined
Surface bound heparin sulphate	High	Released into medium ²⁸	Low ²⁵
		Low	

*Many of these characteristics have been determined for a single or at best only a few cell types. Also, when a particular characteristic has been examined in many cell types the surface changes indicated in this table have at times not been consistently observed.

Implications for growth control

Earlier, using lectins as probes for the examination of the cell surface, we suggested that the cell surface architecture of mitotic cells is similar to that of transformed cells and that this surface configuration might have relevance to growth control¹. Recent data from several laboratories^{3,4,9-26}, summarised in Table 2, indicate in greater detail the striking similarity between the surfaces of mitotic and transformed cells. Such a pronounced correlation alone entices one to speculate about a cell surface involvement in the mechanisms regulating cellular proliferation.

There is much circumstantial evidence which suggests that succinyl-con A is exerting its inhibitory effect through specific binding to the cell surface. The vast majority of processes mediated by lectins, for example, receptor site patching and capping²⁷, cell agglutination, and lymphocyte mitogenesis^{28,29}, involve the binding of the lectin to the cell surface. Two other preparations, glycolipids obtained from *Salmonella minnesota* R mutants³⁰ and dextran sulphate polymers³¹, have been reported

Trowbridge and Hilborn³², since we believe that the binding that is important for growth inhibition occurs during mitosis and in culture conditions. Glucose and serum components bind competitively to succinyl-con A (M.M.B., unpublished) requiring, therefore, higher overall concentrations to reach the effective concentration at the cell surface. It is not surprising that the concentration dependence of succinyl-con A-induced growth inhibition does not agree with studies of con A or succinyl-con A binding.

We have also observed that succinyl-con A can reduce the growth of both polyoma and SV101 transformed 3T3 cells. Because the stimulatory effect of serum on cell growth is antagonistic to the inhibitory effect of succinyl-con A, inhibition of transformed cells induced by succinyl-con A is best observed in the presence of low serum concentrations (2–5%). In these conditions we have observed a marked, non-toxic reduction in the growth rate of these transformed cell lines, although we have not yet observed complete inhibition.

Some of the factors regulating the growth of 3T3 cells are shown in Fig. 3. On entering the mitotic phase of the cell

cycle the cells undergo a change in their surface architecture and receive a "go" signal which sends them into another round of the cell cycle. It is possible that a change in the intracellular level of cyclic nucleotides plays a part in this "go" signal³³, but, since there is no convincing evidence that cyclic AMP or cyclic GMP alterations are causally related to growth inhibition and control, many other mechanisms will have to be considered.

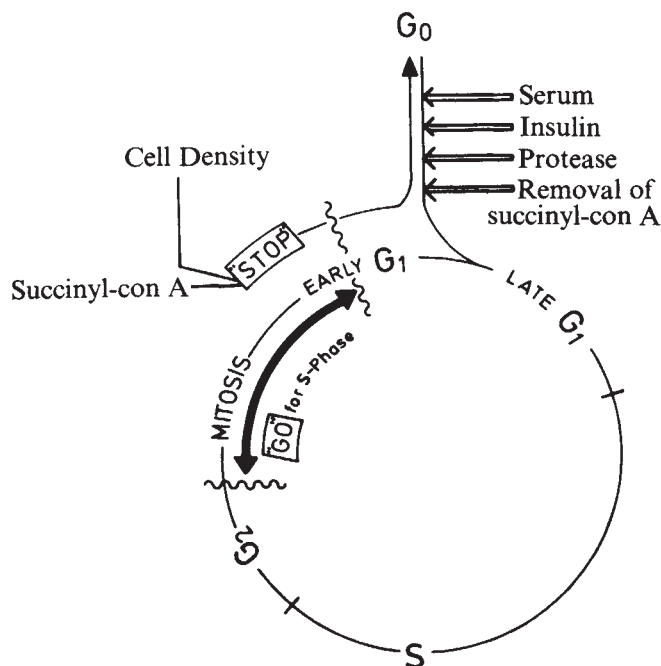


Fig. 3 Proposed interrelationships of various factors regulating the growth of 3T3 cells and the involvement of the mitotic cell surface configuration. The "go" signal occurs early in mitosis, perhaps concomitant with the change to the agglutinable state; the "stop" signal must be received before the cells revert back to the non-agglutinable state some time early in G₁. ↔ indicates the period of the cell cycle during which the cells are agglutinable.

When the cell density in the immediate environment of a given cell reaches a certain level, growth stops and the cell accumulates in G₀, a specific part of G₁. Pardee³⁴ has demonstrated that cells whose growth has been inhibited by high cyclic AMP levels, serum starvation or nutrient starvation, all accumulate at the same point in the cell cycle through a "restriction point" control mechanism. We would hypothesise that cells inhibited by succinyl-con A accumulate at the same point in the cell cycle by the same mechanism.

Cells that have stopped growing and are resting in G₀ can be induced to re-enter the growth cycle by a number of treatments, including serum stimulation, insulin treatment, urea treatment, mild proteolysis and, in the case of succinyl-con A-inhibited cells, removal of succinyl-con A. Serum stimulation, insulin treatment, urea treatment and mild proteolysis all cause the surface membrane to undergo very rapid change to the agglutinable state (Bombik, Bechtel, and M. M. Burger, unpublished, and Weston and Hendricks³⁵). If succinyl-con A is added concomitantly with a serum pulse, cell division is not inhibited, even though the agglutinable surface configuration is known to be present (data not shown). This suggests to us that not only is a specific cell surface configuration required for succinyl-con A to exert its inhibitory effect, but also that the particular surface requirements must occur during a specific part of the cell cycle. We suggest therefore that cells are capable of receiving a "stop" signal

only in early G₁, when untransformed cells still exhibit the cell surface configuration detectable by lectin. If growing cells proceed through this period in the cell cycle without receiving a "stop" signal, they are committed both to a complete round of DNA replication and to cell division. Cells which do not receive the "stop" signal accumulate in G₀ but are "past the point of no return". If G₀ cells are stimulated to re-enter the growth cycle, they are unable to receive a "stop" signal, either from succinyl-con A or from high cell density, until they have proceeded through the entire cell cycle, entered mitosis and once again assumed an agglutinable surface configuration.

The biochemical nature of this "stop" signal is unknown, but two distinct types of mechanisms immediately present themselves. First, succinyl-con A could interact with the cell surface producing a generalised membrane effect, that is, an overall change in the cell surface membrane. Stoker³⁶ has suggested that density-dependent inhibition of growth is the result of a diffusion barrier established when cells grow to high densities; the interaction of succinyl-con A with the cell surface could establish a similar barrier to the diffusion or transport of metabolites essential to growth. Alternatively, succinyl-con A may increase cell surface "stickiness" leading to a decreased mobility after cell-cell collisions and this decreased mobility resulting in growth inhibition (there is no evidence for an increase in the stickiness between cells and substratum).

The second possibility is that succinyl-con A interacts with a specific, unique surface receptor, triggering a series of biochemical events which result in the production of a growth inhibitory "second messenger".

Our data suggest that succinyl-con A interacts with untransformed 3T3 cells during the early G₁ phase of the cell cycle to inhibit growth by a mechanism that, by all the criteria measured so far, seems to be identical to density-dependent inhibition of growth.

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letters to nature

Massive black holes in globular clusters?

FOUR X-ray sources have been identified^{1,2} with globular star clusters (NGC1851, NGC6441, NGC6624 and M15). These are the first X-ray sources to be optically identified with the oldest component (age $\sim 10^{10}$ yr) of the stellar population of our Galaxy; previous identifications of galactic X-ray sources were mostly with bright, young, Population I stars or with relatively recent supernova remnants. Katz³, Clark⁴ and Canizares and Neighbours⁵ have discussed the apparent differences in frequency and population between the globular cluster X-ray sources and other galactic X-ray sources. They have assumed that the X-ray sources in globular clusters are, like the sources identified with Population I stars, binary stars in which a normal companion transfers mass on to a collapsed star, thereby producing X rays.

Here we suggest that the globular cluster X-ray sources may be a very different phenomenon from the previously studied binary X-ray sources. In particular, we suggest that the observed X-ray emission in globular clusters may be caused by accretion of gas on to a massive black hole at the centre of the cluster. This suggestion is consistent with theories of stellar and cluster evolution and with previous observations of globular clusters. Both the theory of cluster core collapse and some observational consequences of black holes in globular clusters have been widely discussed (see refs 6–8 and references therein).

We assume a central massive object accreting at the rate $\dot{M}_{acc}$ and converting rest mass to X rays (2–10 keV) with efficiency ϵ as follows:

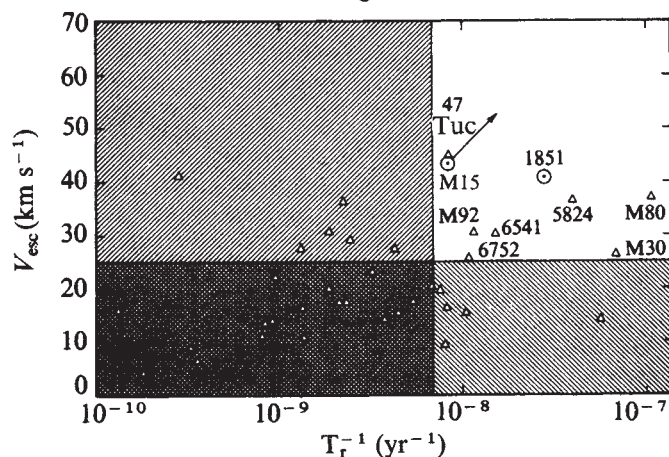
$$L_x = (\epsilon/0.1) (\dot{M}_{acc}/10^{-9} M_\odot/\text{yr}) \times 10^{37} \text{ erg s}^{-1} \quad (1)$$

We have considered two possible sources of accretional mass. Normal stellar evolution in an old system will release

$$\dot{M}_{gas} \approx 10^{-11} M_\odot \text{ yr}^{-1} (L_{cl}/L_\odot) \quad (2)$$

or about $10^{-6} M_\odot \text{ yr}^{-1}$ (with an uncertainty of at least an order of magnitude) in the entire cluster. If this gas is produced

Fig. 1 Globular cluster central escape velocity and central relaxation time from data of Peterson and King¹⁰. Circled points are known X-ray sources. Arrow indicates change for M15 if its core radius has been overestimated by a factor of 1.5. Clusters above hatched regions are expected to be tightly enough bound to retain gas.



with a velocity of 25 km s^{-1} (characteristic of planetary nebula expansion rates) it will be retained in some, but not all, of the globular clusters (Fig. 1). Dimensional arguments suggest⁹ that $T \geq T_{BB} \equiv [GMM_{acc}/8\pi R^3\sigma]^{1/2}$ (where R is the distance from the black hole), which implies that the X-ray efficiency could be appreciable. Massive black holes may also be able to acquire the gas required for accretion directly by tidally disrupting stars which approach too closely. A very rough analysis of this problem gives a rate proportional to $M_H^{4/3} \rho_c^2 r_c^{-1}$ where M_H is the mass of the hole and (ρ_c, r_c) are the cluster core density and radius. Taking observed numbers for these parameters from Peterson and King¹⁰ (with $|M/L|_{cluster}$ assumed 1), we find $\dot{M}_{dis} \lesssim 10^{-9} (M_{BH}/10^3 M_\odot)^{4/3} M_\odot \text{ yr}^{-1}$. This rate is much less than that given by normal stellar evolution but it is produced in the immediate vicinity of the hole and might be accreted with greater efficiency.

If the gas is streaming through the centre of the cluster with a characteristic velocity, v_{gas} , then it will be captured¹¹ at a characteristic radius

$$R_c = GM_{BH}/v_{gas}^2 \approx 2 \times 10^{13} \text{ cm } (M_{BH}/M_\odot) ((25 \text{ km s}^{-1})/v_{gas})^2$$

where M_{BH} is the mass of the black hole. At somewhat larger distances from the black hole, the characteristic density would be

$$n_{gas} \sim (\pi R_c^2 v_{gas} m_{proton})^{-1} (dM/dt)_{accretion} \quad (3)$$

or

$$n_{gas} \sim 15 \text{ cm}^{-3} (L_x/10^{37} \text{ erg s}^{-1}) (0.1/\epsilon) \times (10^3 M_\odot/M_{BH})^2 (v_{gas}/25 \text{ km s}^{-1})^3$$

For a gas with this typical density, 15 cm^{-3} , the total mass, if it were spread uniformly over the central R_{pc} of the cluster, is only $M_{gas} \sim 2 M_\odot R_{pc}^3$. There may also be additional gas not in the cluster core, part of which will eventually fall into the cluster centre.

There is a minimum black hole mass that can produce the observed X-ray intensity, since the mass of gas accumulated in the cluster is less than or equal to $\dot{M}_{gas} t_{encounter}$, where $t_{encounter}$ is the time since the last passage through the galactic disk. The accretion rate, $\dot{M}_{acc} = 4\pi R_c^2 \rho_c v_c$, is proportional to M_{BH}^2 and must be equal to $(L_x/c^2\epsilon)$. The pressure scale height for the gas heated by X rays is $\sim 1 \text{ pc}$ for typical parameters which implies that much of the gas is unavailable for accretion. Assuming an approximate isobaric relationship, $\rho_c T_c = \rho_{core} T_{core}$ (where the cluster core is typically $\sim 0.4 \text{ pc}$) and inserting the maximum allowable value for ρ_{core} ($\sim 0.1 M_{gas} t_{encounter}/\text{volume of the core}$) in equation (3), one finds a minimum black hole mass:

$$M_{BH} \geq 10^{2.5} M_\odot \left| \left(\frac{0.1}{\epsilon} \right) \left(\frac{L_x}{10^{37} \text{ erg s}^{-1}} \right) \left(\frac{v_{gas}}{25 \text{ km s}^{-1}} \right)^3 \left(\frac{T_{core}}{0.1 T_c} \right) \right|^{1/2} \quad (4)$$

Also because of the limited amount of gas available near the black hole, the X-ray luminosity is, for typical parameters, only $L_x \sim 10^{32.5} \text{ erg s}^{-1} (M_{BH}/M_\odot)^2 (M_{gas}/10 M_\odot) (\epsilon/0.1)$, much less than the Eddington limit for the masses we consider (but

comparable with the observed luminosities). Normal cluster stars could have retained close white dwarf companions and accretion onto these might give $L_x \leq 10^{36}$ erg s $^{-1}$, but could not explain a bright source such as NGC6624.

Most of the gas will be ionised by ultraviolet radiation from the stars in the cluster¹² as well as by the X radiation from the vicinity of the black hole. The total mass of ionised gas may be too small to produce any observable absorption in the X-ray spectrum or to have been detected in the most sensitive radio searches conducted so far (typical good upper limits for H II are $\sim 30 M_\odot$ for several clusters¹³; there are stronger limits^{14,15} for H I). The most sensitive possible radio searches for thermal bremsstrahlung from H II gas in the X-ray clusters would be of interest.

We note that a search for Balmer emission lines in the cluster centres would also be of great interest. The expected total flux from the ionised gas is $\sim 2 \times 10^{-13}$ erg cm $^{-2}$ s $^{-1}$ (distance/10 kpc) $^{-2}$ in H β . This is much less than the continuum optical emission from a typical X-ray globular cluster even when one takes into account only that small part of the cluster continuum that would lie within a reasonably small slit. Nevertheless one might, with good luck and excellent technique, be able to place significant limits on, or detect, the postulated gas. Typical expected equivalent widths are of the order of 10^{-2} Å in an H β line of ~ 1 Å width and depend on, among other things, the slit size used and the unknown spatial extent of the ionised gas. The usually strong forbidden emission lines, such as O II $\lambda 3727$ and O III $\lambda 5007$, should be missing (at least near the centre of the cluster) because the typical ionisation stage in the presence of the X-ray flux would be higher.

The model considered here leads to two unambiguous predictions. First, the X-ray emission should be point-like and located at the centre of the globular cluster. Second, there should be no eclipses, orbital or spin periods observed in the X rays (although periods $\sim 0.1(M_{\text{BH}}/10^3 M_\odot)$ s, from particles orbiting near the black hole, might occur in some circumstances). One can show that a pair of massive black holes, or a swarm of lower mass condensed objects, would have evolved dynamically to a single central rotating black hole through many-body interactions and gravitational radiation. One might hope that the observable star distribution and velocity dispersion near the centre of a globular cluster X-ray source are somewhat different than in other clusters. But for an integrable star density $\rho \propto r^{-\alpha}$ near the hole (Peebles⁸ gives $\alpha = 9/4$), one finds a fractional increase in the luminosity of only

$$\Delta L/L_{\text{core}} \approx [\alpha/(3-\alpha)](M_{\text{BH}}/M_{\text{core}})^3 \quad (5)$$

which is probably not observable unless $M_{\text{BH}} \leq 0.2 M_{\text{core}}$. The detection¹⁵ of 10.2 μ m radiation from the centre region of M15 suggests that something unusual is indeed happening in the core of this X-ray cluster. Further infrared measurements of globular clusters are of the greatest possible interest (especially since a large fraction of the total luminosity in the X-ray clusters could be in this spectral region).

Finally we must consider the reasons why, on our model, only a small fraction ($\sim$ a few per cent) of the globular clusters in our Galaxy are X-ray sources. There are at least three obvious possibilities. First, only a moderate fraction of the clusters are bound tightly enough to retain gas. Second, the gas production rate may be sporadic. Third, it may be that only a few of the globular clusters have evolved dynamically far enough to have formed massive black holes in their centres. From a detailed investigation of Spitzer's Monte-Carlo simulation^{6,7}, it seems that core collapse occurs at a time $\tau \simeq 10^3 \tau_c$ from the moment when the central relaxation time is measured to be τ_c . Using data from Peterson and King¹⁰, we show the deduced values of τ_c in Fig. 1. The clusters to the right of the hatched region should collapse within the next 12×10^9 yr. They are correspondingly the best candidates for clusters

within which core collapse has already occurred. It is interesting to note that the two X-ray clusters (shown by circles in Fig. 1) in the well-studied group of Peterson and King lie in the relatively small region having short core relaxation time and large central escape velocity.

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Anisotropic redshift distribution for compact galaxies with absorption spectra

RUBIN *et al.*¹ have found that in one hemisphere of the sky Scl galaxies with $14.0 \leq m \leq 15.0$ have a mean velocity $1,365 \pm 200$ km s $^{-1}$ larger than in the other hemisphere. Anisotropies, corresponding to this, have also been found for brightest cluster galaxies, supernovae (T.J., G. Le Denmat, M.M., J. C. Pecker and J.P.V., unpublished and ref. 2), and Markarian galaxies (H.K. and M.M., unpublished). Here we present a similar result for absorption line compact galaxies.

Zwicky's catalogue³ has been used, selecting from it the compacts with measured redshift and absorption spectra. Since absorption line and emission line compact galaxies have different (m , z) relations these cannot be sampled together, and the number of the latter is rather small for a separate analysis. In absorption spectra also, those with [O II] λ 3727 emission were included in the case that the compact is not blue (most emission objects are blue), since this line is a common component in the normal spectrum of galaxies. Galaxies which are not compact but only have a compact core or some other compact part were not included, for the same reason as the emission objects. In the case of pairs only one component was included in order not to have double weight for these objects, a minority of the sample. The brighter one was taken if m_p is given for each component but if not we corrected m_1 by

0.75 mag as an estimate of the individual m_p . If one of the components was indicated to be brighter than the other, a half correction, 0.37 mag, was applied to m_T . The mean velocity was used in the case of pairs. The cosec law with coefficient 0.25 mag was used for correction of galactic absorption, and galaxies with $|b^{\text{II}}| < 20^\circ$ were excluded. The sky was divided into two regions, as was done by Rubin *et al.*¹ (Fig. 1). As in the earlier studies, the smallest velocities $V < 3,000$ km s $^{-1}$ (only one compact) were not considered. The Hubble modulus $HM = \log V - 0.2m$ is the suitable parameter for this kind of study since it is purely observational and reflects best individual absolute luminosities or, alternatively, individual V/r values in a sample.

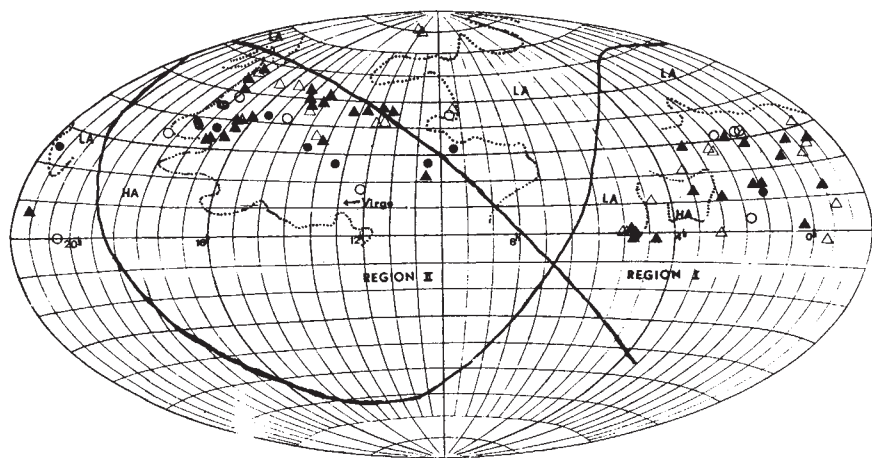


Fig. 1 Distribution of compact galaxies with known redshifts and absorption spectra on the sky. Circles are for galaxies with $m_c < 15.0$ and triangles for those with $m_c > 15.0$. Filled symbols show galaxies with $\delta HM > 0$ and open symbols those with $\delta HM < 0$ where $\delta HM = HM - \langle HM \rangle$, the mean value $\langle HM \rangle$ being calculated separately for intervals $m_c < 15.0$ and $m_c > 15.0$. The full curve from upper left to lower right is the borderline between the two regions of sky. The other full curve shows the galactic equator. The areas included between dashed lines and the galactic equator are those of exceptionally high and low absorption, denoted by HA and LA, respectively. The latter have been transformed from the map in galactic coordinates given by Holmberg⁴, and show areas where absorption deviates from the law $A = 0.25 \csc |b|$ by ± 0.15 mag or more.

Considering the interval of corrected magnitude $14.0 < m_c \leq 15.0$, we obtain for the mean HM in region I the value $\langle HM \rangle_I = 0.88 \pm 0.10$ (5 galaxies) and for region II $\langle HM \rangle_{II} = 1.12 \pm 0.04$ (10 galaxies). For the galaxies with $m_c \leq 15.0$ (all have $m_c > 13.0$), $\langle HM \rangle_I = 0.93 \pm 0.07$ (7 galaxies) and $\langle HM \rangle_{II} = 1.10 \pm 0.04$ (15 galaxies). For the galaxies with $m_c > 15.0$ (all have $m_c < 18.0$), $\langle HM \rangle_I = 0.82 \pm 0.04$ (34 galaxies) and $\langle HM \rangle_{II} = 0.87 \pm 0.03$ (27 galaxies). For the whole interval $13.0 < m_c < 18.0$, $\langle HM \rangle_I = 0.84 \pm 0.04$ (41 galaxies) and $\langle HM \rangle_{II} = 0.95 \pm 0.03$ (42 galaxies). In the different m_c intervals the differences in $\langle HM \rangle$ between regions II and I are 0.24 ± 0.107 , 0.17 ± 0.089 , 0.05 ± 0.050 and 0.11 ± 0.050 , respectively. These are significant at 2.24σ , 1.91σ , 1.00σ , and 2.20σ levels, corresponding to chance probabilities 0.020, 0.035, 0.170 and 0.015. The mean difference of velocities of objects in region II and I is $3,400 \pm 1,850$ km s⁻¹ for $m_c < 15$ and $700 \pm 1,400$ km s⁻¹ for $m_c > 15$. For $m_c < 15.0$ the average values $\langle m_c \rangle = 14.3$ and $\langle V \rangle = 8,650$ km s⁻¹ are valid. For $m_c < 15.0$, $\langle m_c \rangle = 16.1$ and $\langle V \rangle = 12,700$ km s⁻¹.

From the differences in $\langle HM \rangle$ one finds that compact galaxies with absorption spectra show a significant redshift anisotropy in the same direction as previously found^{1,2}. The anisotropy decreases from the maximum at $14 < m_c \leq 15$, especially towards fainter magnitudes. This favours the view that the effect is not due to galactic or intergalactic absorption since absorption would also cause an apparent anisotropy at large distances. This view is supported also by Fig. 1 which gives the distribution of the galaxies on the sky and information on individual HM and shows the regions of exceptionally high and exceptionally low absorption as given by Holmberg⁴. Small HM are not associated with areas of high absorption and large HM with areas of low absorption, as would be expected for an apparent anisotropy attributable to absorbing clouds⁵.

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Pulsar glitches and restlessness as a hard superfluidity phenomenon

SEVERAL pulsars¹ have "restless" behaviour of the period similar to that of the Crab and Vela pulsars² which has been explained³ as due to "microquakes" in the crust. A glitch has also been observed¹ with, possibly, a new type of signature, in PSR1508+55. We suggest that these phenomena can be explained at least equally well by noisiness in the rate of creep of vorticity through the neutron superfluid in the crust, in almost precise analogy to the well known noisy behaviour of flux creep in hard superconducting magnets⁴. In addition, even the macroglitches, especially those in the Vela pulsar, may be caused by the catastrophic release of pinned vorticity.

The lower crust of the neutron star contains fat nuclei bathed in a neutron Fermi gas which is almost surely superfluid⁵. The discussions we have found of this superfluid do not observe that its T_c and energy gap will at most densities be determined by the proximity effect from those of the neutron superfluid inside the nuclei, since the outer Fermi gas is from $1/2$ to $1/10$ as dense and will probably have a smaller intrinsic gap (see Fig. 1 and ref. 6). The neutrons inside the nuclei will contribute to some extent to the neutron ρ_s , since their phases are coupled by Josephson tunnelling through the surrounding gas even if that gas is not intrinsically superfluid. T_c s and Δ s in the $1/2$ to 2 MeV range are reasonable, as is a $\rho_s \sim 10^{12}$ – 10^{14} g cm⁻³. Because of the tunnelling effect the correlation length will usually be essentially the internuclear distance: the substance is a very hard Type II superfluid⁴.

As Pines pointed out, pinning of the quantised vortex lines which are implied by the rotation of the superfluid,

$$n_{\text{vortices}} = 2m\Omega/\hbar \sim 10^{4-5} \text{ cm}^{-2} \quad (1)$$

can occur in the crust; they will to some extent be pinned by the regular lattice, since the coherence length is never much greater than the internuclear distance, and even more so by cracks, grain boundaries and other features such as are surely created by quakes. We visualise that vortex lines can slide along lattice planes relatively easily, and are blocked especially severely at these features. Experience with pinning in the analogous hard superconductors suggests that the breaking strength of this vorticity pinning may be surprisingly high. To estimate the pinning strength we estimate the energy of a vortex core as

$$E_{\text{core}}/\text{length} = (\Delta^2/E_F)(k_F^3 \xi^2)$$

and the force per unit length exertable by pinning is this

divided by the distance over which the force acts,

$$F/\text{length} = (\Delta^2/E_F)k_F^3 \zeta \times p \approx k_F^3 \Delta p \approx 10^{20} p \text{ dyne cm}^{-1} \quad (2)$$

where p is a "pinning probability" factor < 1 describing the fractional density and effectiveness of pinning centres (comparable to the factor p in refs 7 and 8). This force is also of the order one would estimate for the strength of the lattice itself, so that vorticity may fracture the structure rather than

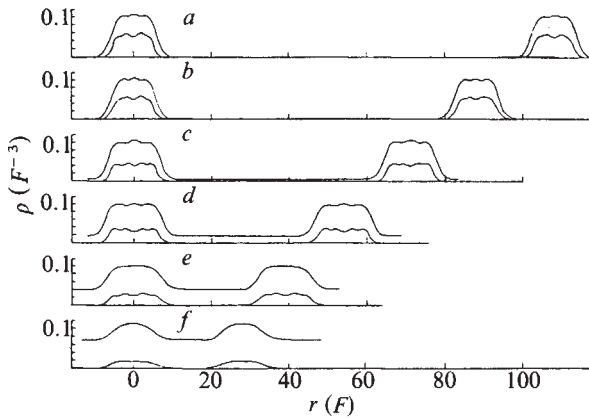


Fig. 1 Proton and neutron density distributions occurring along an axis joining the centres of two unit cells. *a*, $^{180}\text{Zr}_{40}$ with $n_b = 2.79 \times 10^{35}$; *b*, $^{320}\text{Zr}_{40}$ with $n_b = 8.79 \times 10^{35}$; *c*, $^{1100}\text{Sn}_{50}$ with $n_b = 5.77 \times 10^{36}$; *d*, $^{1800}\text{Sn}_{50}$ with $n_b = 2.04 \times 10^{37}$; *e*, $^{1500}\text{Zr}_{40}$ with $n_b = 4.75 \times 10^{37}$; *f*, $^{982}\text{Ge}_{32}$ with $n_b = 7.89 \times 10^{37}$.

unpin from the lattice. Equation (2) must be equated with the Bernoulli force on a vortex:

$$F = (h/2m)p\nu \text{ per unit length} \quad (3)$$

to obtain a critical relative velocity of crust and neutron superfluid at a given point

$$\begin{aligned} v_c &\approx (2m/h\rho)k_F^3 \Delta p \\ &\approx 10^8 p \text{ cm s}^{-1} \end{aligned}$$

If the difference in angular frequency $\Omega_c - \Omega_n$ is $\Delta\Omega$,

$$(\Delta\Omega)_{\text{crit}} = v_c/R \approx 50 p \text{ s}^{-1} \quad (4)$$

Thus even if p is very small there can be strong pinning forces. If it is the actual lattice which pins, p will not be particularly small, $\sim 1/10$ – $1/100$ or so. But equation (4) indicates such large values of $(\Delta\Omega)_{\text{crit}}$ that we must expect that in most stars the superfluid in the crust is effectively pinned and does not change its Ω , and that in others it will be pinned in some regions and at some times. The former are probably the majority of pulsars which show quiet behaviour¹.

Here we give some suggestions, necessarily rather speculative, as to the actual physics of superfluidity in the pulsar. In the first place, the frictional force suggested by Baym and Pines due to electron scattering on vortex cores is far too small to unpin the vortices; that force may be estimated to be

$$\begin{aligned} F_{\text{friction}} &= \rho R \Delta\omega / \tau \text{ per unit volume} \\ &\lesssim 10^5 \text{ dyne cm}^{-3} \end{aligned}$$

where τ is the spin-up decay time of order months introduced by Baym *et al.* If the Bernoulli force due to relative motion of

crust and superfluid is not larger than indicated by equation (2) the vortices stay at fixed positions in the crust rather than flowing with the superfluid as they do in a normal soft superfluid. The Bernoulli force is strong enough that the vortex lines in the core fluid will probably not move with the crust, but will flow with the core superfluid. This must lead to complicated dynamics at the interface; our suspicion is that a turbulent boundary layer will form which breaks up all phase coherence at the crust–core boundary. The theory of these phenomena has been discussed by Greenstein⁹. As he points out, the viscosity due to the turbulence can be of the same order as that due to electron scattering and can be the cause of spin-up. The turbulence and pinning seem likely to damp out any Tkachenko waves or similar collective modes of the vortex lattice. In any case, vorticity need not be perfectly conserved in passing from core to crust, as one might have thought at first.

Thus the dynamics of the core and of spin-ups is little affected. But what does appear here is a second, decoupled, superfluid which in the case of light stars may be a fair fraction (10^{-2} – 10^{-1}) of the neutron superfluid. This crustal superfluid may lag behind the slowing angular velocity of the crust by some cycles per second. The slowing of this superfluid must necessarily take place by the very noisy process of vorticity creep, with concomitant "vorticity jumps" which occur when a particular group of pinning centres become too weak, and may be thought of as the superfluid vortex lattice's equivalent of crustquakes. The reason why these processes will be noisy is that once vortex lines start to move, there is no adequate source of damping to soak up the rotational kinetic energy which is thereby deposited in the vortex lattice. Whether the vortex lattice or the crust itself fractures is not relevant; in either case the event must continue until the stress is relieved.

A second, somewhat independent consequence of superfluid dynamics in neutron stars is the fact that even in the "canonical" view the superfluid never rotates with the same angular velocity as the pulses or the crust, but lags behind by a relative amount τ/T (T is the "lifetime" parameter of ref. 1). The relative lag of crustal superfluid is much greater. It is not obvious that in all cases these angular velocities should be coaxial; this should only be the case if the forces are always simply drag forces and have no couple perpendicular to the axis. In an accreting star such as Her X-1 the accreting matter can exert non-axial couples, and one would not expect Ω_c and Ω_n to be parallel. This can lead to further complications in the dynamics of such systems.

One may estimate that the amount of energy stored in the vortex cores is only $\lesssim 10^{-12}$ of the total rotational energy of the neutron star: using the estimate already given one cannot assume much more than 10^{30} erg in a shell 1 km deep at a radius of 10 km. Nonetheless the associated events may, just as in a superconducting magnet, be much larger than the energy involved in pinning. It is the stored relative rotational energy which is relevant, which can easily be 10^{41} erg. This can be larger than the elastic energy which is responsible for quakes, while the magnitude of strength parameters is similar; thus these events will tend to be larger and more frequent, at least in light stars, than quakes. Thus any of the observed events seem explicable on this basis. We do, however, consider it likely that at least crust quakes will occur as well, and that sometimes the two can occur together.

Superfluidity and hydrodynamic instability mechanisms previously suggested for glitches^{9,10} are quite distinct from the present one, and, we believe, less likely. A matter of some importance is that the suggestions for laboratory simulation of the rotating superfluid star become even more meaningful, but that the correct model should have a porous outer layer: the model should be a Reppy superfluid gyroscope¹¹ with a hole in the centre, not simply a ball of superfluid. Such experiments could help with the vexing question of the boundary layer viscosity and the continuity of vortex lines from core to crust. It would be interesting to study vorticity jumps in such a structure as well as precessional motions.

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Giant olivine chondrule as a possible later-stage product in the nebula

ABUNDANCES of trace elements, in particular, of rare-earth elements (REE) in the Allende Chondrite and its inclusions have attracted increasing attention from geochemists¹⁻⁴. Based on the abundance characteristics of major elements and trace elements (including REE), Martin and Mason⁵ distinguished four distinct groups among the aggregates and chondrules of the Allende Meteorite. We report here on the presence of an olivine chondrule in Allende with REE abundance features quite different from those of any inclusions previously reported.

By chance, we found an extraordinarily large chondrule in Allende (Fig. 1). It measured 7 mm in diameter and appeared light grey with darker coloration towards the margin. In general, the olivine chondrules in this meteorite are only a few millimetres or less in size: previously Tanaka and Masuda¹ studied Allende olivine chondrules which had diameters of about 3 mm. Chemical analysis and X-ray diffraction indicate that the composition of this giant chondrule is much closer to pure olivine than that of the olivine chondrules analysed before⁴. Chemical

Fig. 1 A giant olivine chondrule in the Allende Chondrite. Scale bar, 1 cm.

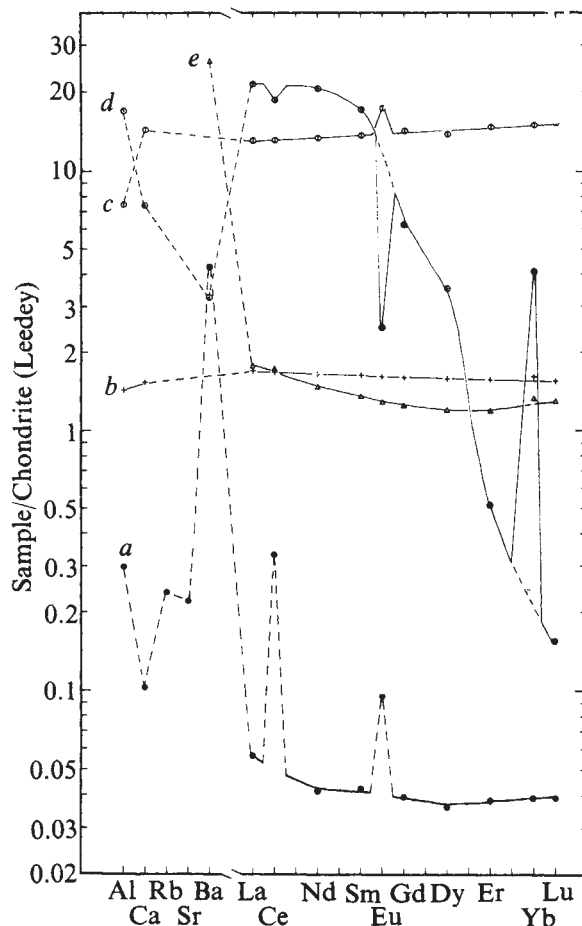
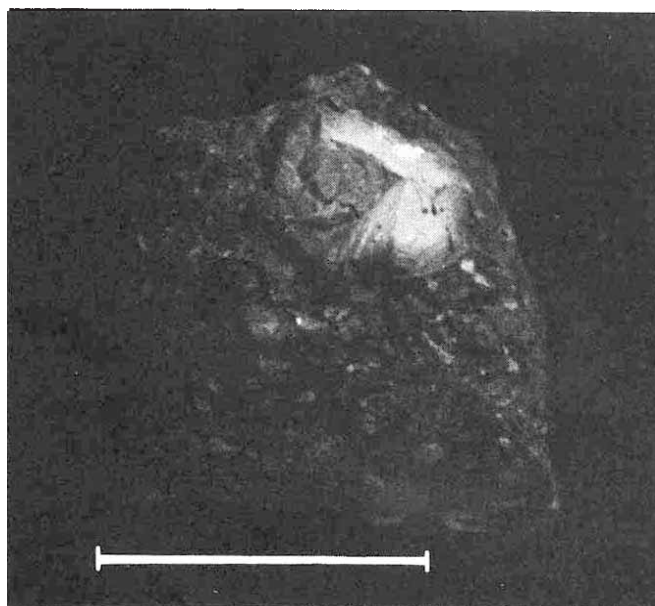


Fig. 2 Chondrite normalised pattern using the Leedeey Chondrite as standard. a, REE and other elements from the giant olivine chondrule in the Allende Chondrite; b-e, other inclusions in the same meteorite; b, olivine chondrule; c, Ca, Al-rich chondrule; d, inclusion G pink; e, whole rock.

analyses by Nakamura *et al.* (unpublished) suggest that the material studied here is almost pure olivine (Fo_{70}) with perhaps 1.5% feldspathic or glass component.

About 120 mg of the sample were used for determination of REE, Ba, Sr and Rb abundance using a stable isotope dilution technique; Ca and Al abundance were determined by atomic absorption spectroscopy. Analyses are presented in Table 1 and Fig. 2. (For the normalisation of REE abundances, the Leedeey chondrite⁵ is used as a reference.) The abundances of common REE in the giant olivine chondrule are 40 times lower than those in ordinary olivine chondrules, and there are positive anomalies for Ce and Eu. Although a Yb anomaly is often observed in meteorites^{1-3,6}, it is not present in this sample. The abundance of Ba is 4 times as high as the reference value, whereas that of Sr is about half the reference value and that of Ca is as low as one-tenth of the corresponding chondritic reference abundance. There is no comparable fractionation among alkaline earth metals in ordinary olivine chondrules^{1,3}. The extent of the fractionation of Ba, Sr and Ca may be more or less related to the absolute levels of abundance of common REE and also, presumably, to the size of the olivine chondrule. In addition, a considerable depletion of Al also distinguishes the giant olivine chondrule from ordinary olivine chondrules¹. In the giant chondrule the contents of Na and K (Nakamura *et al.*, unpublished) are about one-third to one-fifth of those in the Allende bulk sample². Thus, the olivine chondrule studied here is extraordinary not only in size, but also in its chemical features.

It is conceivable that the material constituting the giant olivine chondrule came into being in conditions which favoured

Table 1 Abundances of REE, Ba, Sr, Rb (p.p.m.), Ca and Al (%) in a giant olivine chondrule from the Allende Chondrite

La	0.021	Gd	0.0122	Ba	18.0
Ce	0.32	Dy	0.0140	Sr	2.44
Nd	0.029	Er	0.0098	Rb	0.67
Sm	0.0098	Yb	0.0097	Ca	0.129
Eu	0.0082	Lu	0.00152	Al	0.33

the precipitation of material with an olivine composition only, though these conditions would not have precluded the precipitation of material which gave rise to other chemically different types of chondrules. The very low abundances of common REE imply that these refractory elements had been depleted from the gaseous phase of the nebula by the time that the material represented by this giant olivine chondrule had formed.

The positive Ce and Eu anomalies can be accounted for by the relatively high pressures of the oxides of Ce and Eu^{7,8}. Oxides of Ba, Ce, and Eu are more volatile than those of other common REE, and the order of volatility of the metal oxides is Ba > Ce > Eu (ref. 9).

It is worth considering the low content of Rb, one of the comparatively volatile elements. It is possible that although the giant olivine chondrule formed as a later-stage product in the nebula, the temperature at that stage was not low enough to condense the alkali metals in the chondrule.

The characteristics observed in the chondrule are not entirely in line with earlier thermodynamic studies of REE fractionation¹⁰. (Note the presence of a positive large Ce anomaly, the absence of a positive Yb anomaly, and a mutually unfractionated, flat pattern for common REE from La to Lu.)

In any case, we suggest that the giant olivine chondrule is extremely pure material which could have formed at specific temperature ranges. Broadly speaking, this chondrule of wholly new type may be complementary to Ca, Al-rich aggregate with unfractionated REE (ref. 3) though, in detail, additional components are required.

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and therefore may be interpreted as indicating the presence of anomalously warm material only a few tens of kilometres beneath the Earth's crust. We believe these observations support the hypothesis of an incipient arm of the East African rift system as proposed by Fairhead and Girdler^{1,2} and suggest that it may extend into the central African plateau to at least 16°S.

Temperature surveys were made with thermistor probes in more than thirty boreholes distributed over the eight sites, usually several weeks to several months after drilling had been completed. Depths of measurement range from 160 to 1,200 m. Thermal conductivities of solid rock disks sampled from drill cores at 10 to 20 m intervals were subsequently determined on a conventional divided bar apparatus. Heat flow values were then calculated as the product of the least squares geothermal gradient and the harmonic mean conductivity. Mean heat flow values for the sites are given in Table 1.

The results of radioactive heat production measurements on aggregate samples of core chips from several of the sites are also presented in Table 1. No heat production values were obtained for the Ichimpe and Luanshya sites where the boreholes penetrate mainly sedimentary sequences.

In interpreting continental heat flow results it is important to ascertain the tectonic and thermal history of the region. Apart from limited areas covered by Karroo (Permian-Jurassic) sedimentary and volcanic rocks and Kalahari (Pleistocene-Holocene) sands, most of Zambia consists of pre-Silurian, mainly Precambrian, rocks³ (Fig. 1). The last tectono-thermal event recorded in Zambia is the Damaran-Katangan (Pan-African) episode, which is represented at the northern heat flow sites by isotopic ages of 520±50 Myr, and at the southern sites by ages of 730±50 Myr, (ref. 3). One site, Mkushi, has been unaffected since the earlier Kibaran orogeny and has been dated⁴ at >1,635 Myr.

Figure 2 is a graph of continental heat flow as function of age of most recent tectonic mobilisation, after Polyak and Smirnov⁵, who identified in some detail the clear trend of diminishing heat flow with increasing age of tectonic province. Also shown in Fig. 2 is the mean value of 66 mW m⁻² for the eight Zambian sites, plotted collectively at 700 Myr. The Zambian surface heat flow is anomalously high by about 30 mW m⁻² compared with Precambrian shields elsewhere.

Large surface heat flow can result merely from enhanced radiogenic heat production in the upper few kilometres of the crust. We have compared our heat production measurements with all available data from other Precambrian shields and conclude that enhanced heat production can account for no more than one half of the 30 mW m⁻² surface anomaly. We therefore infer a heat flux anomaly of at least 15 mW m⁻² originating in the lower crust or below. Furthermore, as lower crustal and upper mantle rocks are depleted in heat producing isotopes by a factor of five to ten relative to upper crustal rocks, it is unlikely that the source of the residual heat flow anomaly is radiogenic. A more probable source is an anomalous zone of elevated temperatures at depth resulting from an intrusion or from some thermal process in the asthenosphere.

We consider two interpretive models to explain the observed heat flow anomaly. The first involves the computation of surface heat flow in time following a sudden increase in temperature at a given depth. In the plate tectonic model this may correspond to the lithosphere coming to rest over an asthenospheric hot spot or a convective upwelling. If such an event happened during the Miocene⁶, plausible solutions restrict the depth of the disturbance to less than 60 km; at greater depths an unacceptably large temperature perturbation is required to produce the observed surface anomaly. A second model involves a temperature perturbation which is spatially

Heat flow and incipient rifting in the Central African Plateau

EIGHT new heat flow measurements from Precambrian sites in the Republic of Zambia (Fig. 1) range between 55 and 76 mW m⁻². Compared with the mean for Precambrian provinces elsewhere, these results are anomalously high by some 50%. This heat flow anomaly persists after taking into account radioactive heat generation of near surface rocks,

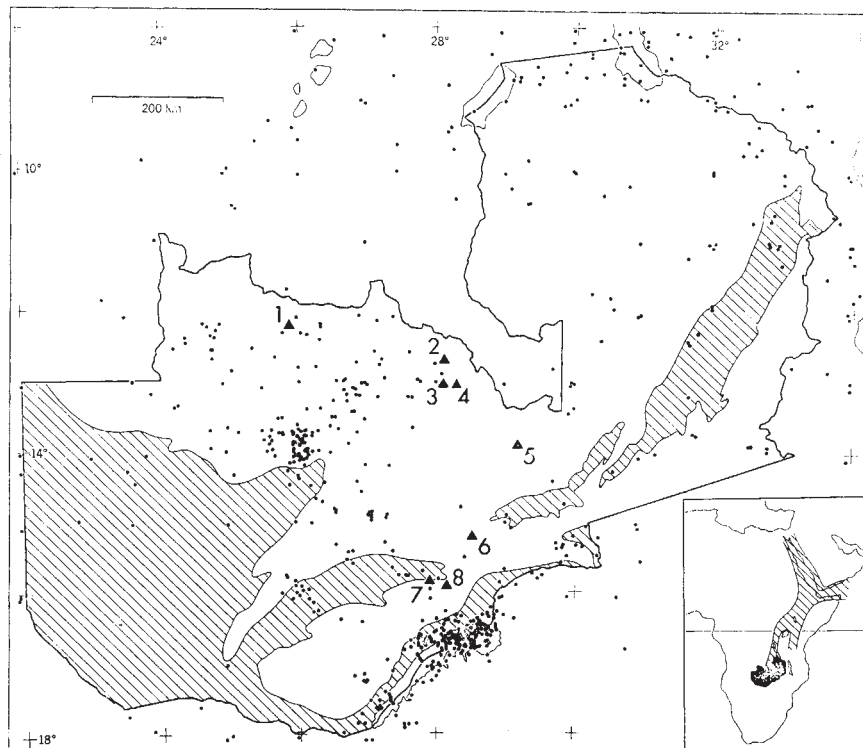


Fig. 1 Location map of the Republic of Zambia showing heat flow sites (▲), earthquake epicentres (●), Precambrian (unshaded) and post Pan-African (shaded) terrains. Epicentres drawn from International Seismological Centre (Edinburgh) Regional Catalogue of Earthquakes, 1965–70. Inset map shows the zone of inferred lithospheric thinning from Fairhead and Girdler².

periodic at the base of a moving slab, corresponding to the lithosphere moving over an array of hot and cold spots below. In this model the solution is an upward propagating thermal wave which is both attenuated and phase shifted at the surface. The magnitude of the observed heat flow anomaly places restrictions on both the depth to the temperature perturbations and the velocity at which the plate moves over them. To satisfy the observed surface heat flow anomaly, calculations utilising this model require that the anomalous zone be less than 60 km deep, and also that the lithosphere be moving less than 2 cm yr^{-1} relative to the source pattern.

Arguments can be made for the applicability of either model. It remains unclear whether the central African region of the African plate is at rest in a hot spot frame of reference⁶, or is moving slowly northwards⁷. In either case the source of the thermal anomaly cannot lie deeper than 60 km, and if the source is an intrusion from, or the upper boundary of, the asthenosphere, it represents a considerable penetration into or thinning of the continental lithosphere.

Lithospheric thinning has already been proposed by Fairhead and Girdler for the tectonically active East African rift system, to explain slow seismic wave propagation and

high attenuation, and large negative Bouguer gravity anomalies. They map a single zone of thinned lithosphere southward from Ethiopia almost to the Equator where it bifurcates into the eastern and western rifts (Fig. 1, inset). The southernmost part of the western zone departs from Lake Tanganyika, and strikes SSW across the topographically unbroken central African plateau, to approximately 12°S .

How far south does this lithospheric thinning extend? The region of high heat flow in Zambia lies directly on the extension of the western arm of the anomalous zone. We therefore believe the geothermal measurements argue convincingly for an extension of the zone of lithospheric thinning southward through western Zambia, at least to 16°S . Some additional support for this interpretation is drawn from the distribution of seismicity in Zambia (Fig. 1). Two clusters of epicentres are apparent; one in the middle Zambezi Valley, a Mesozoic rift structure which has been seismically active since the impoundment of Lake Kariba, and a second in west-central Zambia. The occurrence of earthquakes in the latter region is curiously uncorrelated with any obvious geological or topographic feature, but does lie nearly wholly within the region of high heat flow and conjectured thin lithosphere. The presence of earthquakes

Table 1 Heat flow and heat production data

Site	Latitude	Longitude	Heat production* ($\mu\text{W m}^{-2}$)	Heat flow (mW m^{-2})
1 Lumwana	$12^\circ 15'\text{S}$	$25^\circ 51'\text{E}$	2.5	55 ± 8
2 Ichimpe	$12^\circ 44'\text{S}$	$28^\circ 07'\text{E}$		74 ± 4
3 Lumpuma	$13^\circ 05'\text{S}$	$28^\circ 03'\text{E}$	1.8	65 ± 4
4 Luanshya	$13^\circ 05'\text{S}$	$28^\circ 19'\text{E}$		76 ± 4
5 Mkushi	$13^\circ 55'\text{S}$	$29^\circ 12'\text{E}$	1.9	62 ± 2
6 Chalalobuka	$15^\circ 13'\text{S}$	$28^\circ 33'\text{E}$	2.3	66 ± 3
7 Lubombo	$15^\circ 49'\text{S}$	$27^\circ 55'\text{E}$	3.6	71 ± 2
8 Munali Hills	$15^\circ 55'\text{S}$	$28^\circ 08'\text{E}$	1.0	64 ± 4

*Heat production determinations are subject to measurement uncertainties of about 1%. Uncertainties arising from sampling are limited to less than 15% by assembling aggregate samples from throughout each borehole.

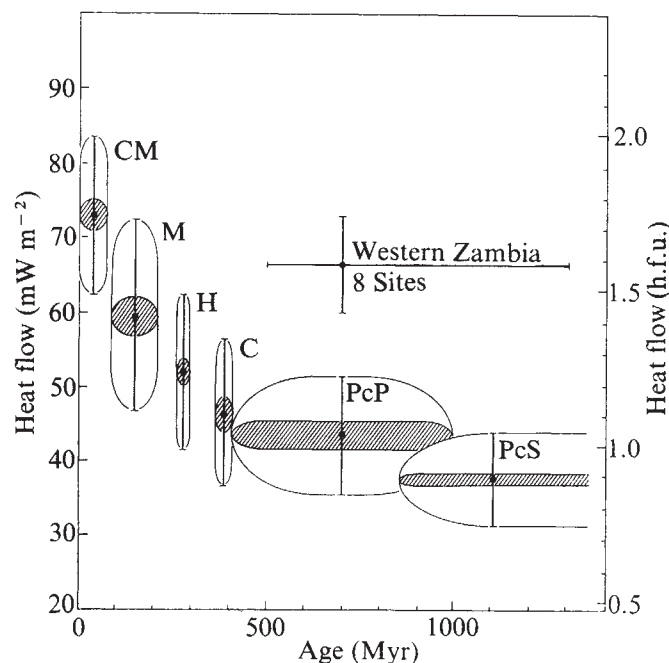


Fig. 2 Mean heat flow from eight Zambian sites superimposed on plot of heat flow against age of tectogenesis after Polyak and Smirnov⁶. Tectonic provinces include: PcS, Precambrian shield; PcP, Precambrian platform; C, Caledonian folding; H, Hercynian folding; M, Mesozoic folding; CM, Cainozoic miogeosyncline. Shaded ellipse represents standard error of the mean heat flow; vertical bar and open ellipse represent standard deviation.

in the anomalous zone suggests that this region, like the mature rifts in East Africa, is dynamic and responding to contemporary tectonic stress.

Further work is required to delineate the boundaries and continuity of this important geophysical anomaly. But our heat flow results to date and the seismicity of western Zambia lend strength to the hypothesis of incipient rifting in the central African plateau.

This project was initiated while we were at the University of Zambia. We thank our colleagues there, and in the Geological Survey of Zambia, Nchanga Consolidated Copper Mines, Roan, Consolidated Mines and Mkushi Copper Mines for assistance. Professor R. F. Roy made the radiogenic heat production measurements. Financial support was provided by the universities of Zambia and Michigan, and the US National Science Foundation.

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Structure of turbulent boundary layers at maximum drag reduction

WE describe here the results of a preliminary study on boundary layers carried out with an aqueous equimolar solution of cetyltrimethylammonium bromide (CTAB) and 1-naphthol at a total concentration of 508 parts per million

(p.p.m.). Dilute, high polymer solutions in pipe flow can produce a drag reduction which is bounded by a limiting asymptote curve¹, and micellar solutions of CTAB-1-naphthol exhibit drag reduction described by the same asymptote^{2,3}. Measurements of the velocity profiles of the micellar solution across a pipe diameter show fairly good agreement with the ultimate profile deduced⁴ from pipe friction results. This particular drag reducing solution is suitable for experiments in closed loop systems because of its resistance to permanent mechanical degradation.

The measurements reported here were taken at the base of a small flume using the pulsed hydrogen bubble technique for flow visualisation and qualitative measurement. Essentially, the method is that developed by Kline *et al.*^{5,6}.

The working section of the flume was 0.28 m wide by 0.20 m deep and was fed from a large stilling chamber, through a contraction, to produce a uniform flow. The circulation was induced by a variable speed centrifugal

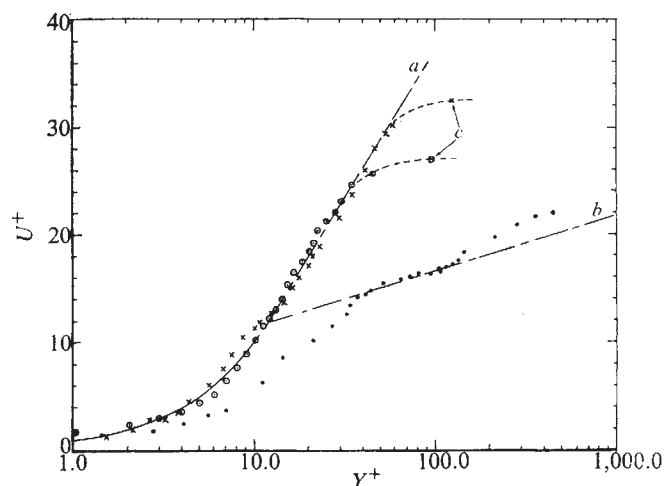
Table 1 Summary of near-wall observations

	Water	CTAB-1-naphthol solution	
U_{∞} (m s ⁻¹)	0.365	0.350	0.250
u^* (m s ⁻¹)	0.017	0.013	0.0077
ν (m ² s ⁻¹)	1.130×10^{-6}	1.280×10^{-6}	1.270×10^{-6}
F (burst s ⁻¹ m ⁻¹)	290	20	9.0
λ (m)	0.0073	0.037	0.050
λ^+ ($= \lambda u^* / \nu$)	115	375	300
T_b ($= 1/F\lambda$)	0.475	1.35	2.22

pump. A boundary-layer trip wire, 1 mm in diameter, was placed across the floor of the channel at the entry end. The stainless steel cathode wire from which the hydrogen bubbles were liberated was 0.05 mm in diameter and was mounted transversely to the flow on a traversing mechanism situated 0.64 m downstream from the boundary-layer trip wire. Flow patterns were recorded on video tape for subsequent analysis.

Mean velocity profiles in dimensionless form are shown in Fig. 1. The wall shear stress, for the micellar solution was determined from the near-wall velocity profile slope. The water test did not provide sufficient data points within the extremely thin viscous sublayer and in that case a modified Clauser procedure was used to determine the wall shear-stress⁷. Experimental results from the micellar

Fig. 1 Boundary layer velocity profiles: $U^+ = u/u^*$; $Y^+ = yu^*/\nu$ (where u is the mean velocity at distance y from the wall; $u^* = (\tau_w/\rho)^{1/2}$; τ_w = wall shear stress; ρ = fluid density; ν = kinematic viscosity). ●, Water, ($u^* = 0.017$ m s⁻¹); ○, CTAB-1-naphthol solution ($u^* = 0.013$ m s⁻¹); ×, CTAB-1-naphthol solution ($u^* = 0.0077$ m s⁻¹). a, $U^+ = 11.7 \ln Y^+ - 17.0$; b, $U^+ = 2.44 \ln Y^+ + 4.9$; c, free-stream values.



solution seem to be adequately described by Virk's limiting-velocity profile model¹.

The bulk of turbulence production is associated⁶ with low speed streaks which originate near the wall in the viscous sublayer and with their subsequent eruption or 'bursting'. From a study of the video records, measurements of the mean transverse spacing of the streaks, λ , the burst frequency per unit span, F , and the time between bursts, T_b , were determined (Table 1). The results obtained with water show fairly good agreement with previous investigations⁵⁻⁷. In addition to producing a large friction reduction the micellar solution showed a large increase in the transverse spacing of the streaks and a large reduction in the burst frequency. These changes are more dramatic than those found by previous investigators who used less effective drag reducing solutions⁷. Although the time between bursts T_b , is increased, the value still seems to correlate with the friction velocity, u^* , on the same unique line as that found with water and other polymeric solutions (ref. 7, and B. U. Achia and D. W. Thompson, unpublished).

The shape of the mean velocity profile supports the use of similarity laws for the prediction of boundary-layer skin friction in conditions of maximum drag reduction.

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Carbon fibre pH-sensitive electrode

VARIOUS forms of carbon have been considered as possible indicator electrodes for use in locating end points in acid-base titrations; these forms have included graphite¹, vitreous or glassy carbon² and pyrolytic graphite³. Microelectrodes can be used in titrations of small volumes, such as those found in biological cells⁴, and certain forms of carbon such as glassy carbon, are exceptionally resistant to oral and tissue fluids⁵. We have examined the electrical potential response of a single carbon fibre to pH changes in aqueous electrolyte solutions.

The potential of a carbon fibre electrode 1 cm long and 7–8 μ m in diameter, sealed into a Pyrex glass tube with epoxy resin, was measured against a saturated calomel reference electrode in various buffer solutions in the pH range 1–13, using a Corning EEL 109 digital pH meter used in its millivolt measuring mode. We found a linear relationship between the potential and pH, with a negative slope of about 50 mV per pH unit at room temperature (about 20 °C). The potential in any given buffer, such as 0.05 M potassium hydrogen phthalate, is stable to within 1 mV, within measuring times of a few minutes. Larger variations in potential (and also the slope factor) were, however, observed when the same electrode was examined over a period of weeks; this implies that a carbon-fibre pH electrode of this kind must be calibrated on each day of its use.

The potential of a carbon fibre pH electrode is unaffected by the presence or absence of dissolved oxygen: the same potential was recorded even when pure nitrogen was

bubbled through the buffer solution. A small negative shift in potential was, however, observed with temperature increase (about 0.6 mV °C⁻¹). The potential of a carbon fibre electrode is affected by the presence in solutions of strong oxidants such as bromine and cerium(IV) and also by the presence of reductants, such as arsenic(III).

We believe that the observed pH response of the carbon fibre electrode may be caused by the possible ionisation of carboxylic acid groups formed on the surface of carbon by surface reactions with atmospheric oxygen^{6,7}.

We thank the carbon fibres unit of Courtaulds Limited, Coventry, for the gift of the low temperature carbon fibres.

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Electrostatic energy of columbite/ixiolite

A KNOWLEDGE of the crystal structure of an inorganic compound and the appropriate ionic charges is usually sufficient to allow the calculation of the electrostatic energy¹⁻³. A more difficult situation is found in crystals where some or all of the cations are disordered so that on the average a particular cation site is occupied by two or more cations with different charges. Very accurate crystal structure refinements, often including refinement of site occupancies, can give a good description of the nature of the disorder and the average composition of each cation site. The electrostatic energy can then be calculated using the average ionic charges on each of the disordered sites. Such an approach has been used to compare the stabilities of ordered and disordered structures of pyrrhotite^{4,5} and some complex niobium and tantalum oxides⁶. There seems no theoretical justification for the use of average cation charges for the calculation of the potential energy of a disordered crystal so such calculations must be treated with caution.

Consider a hypothetical crystal structure in which two different cations with different ionic charges occupy four cation sites such that, in the completely ordered arrangement, three are occupied by one ion (A) and the other site contains the second type of ion (B). The ordered arrangement then may be represented by the symbols AAAB. A completely disordered version of this structure determined from a diffraction experiment would be (as indicated above) 3/4A+1/4B for each of the four cation sites. But on the level of an individual unit cell, each cation can be either A or B only and not some fictitious average ion. There are four unique arrangements of 3A and 1B over four sites, namely AAAB, AABA, ABAA and BAAA and the electrostatic energy of each of these ordered arrangements may be calculated by standard procedures. As we do not know how to calculate correctly the energy of the disordered structure, we must deal with what is available; the energies of all the possible ordered arrangements of the cations.

The mineral columbite, $MnNb_2O_6$, offers a good example. In the orthorhombic unit cell are 12 cation sites occupied by 8 Nb and 4 Mn. The charges on the sites in the ordered structure (columbite) are either 5 or 2 and in the disordered structure (ixiolite) the average cation charge on all sites is 4. Barker and Graham⁶ have calculated the electrostatic energy of columbite and ixiolite and find that the ordered arrangement is very much more stable than the disordered. This is difficult to reconcile

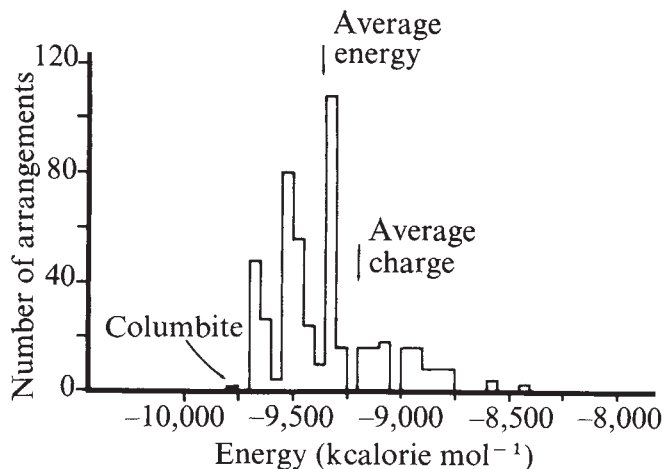


Fig. 1 A histogram of the electrostatic energies of the 495 possible arrangements of 8 Nb and 4 Mn ions in the unit cell of columbite. The "ordered charge" corresponds to the known distribution of the cations, "average charge" represents the energy computed for a structure with average ionic charges (+4) on all cations and "average energy" is the average of all 495 energies.

with the well known tendency of natural columbites to be partially or totally disordered. I suggest that this apparent inconsistency between observation and the calculated energies is due to the incorrectness of the assumption that an electrostatic energy based on average cation charges is an accurate measure of the energy of a real disordered structure.

There are 495 different ways of distributing 8 Nb and 4 Mn over 12 cation sites and the electrostatic energy of each of these structures was calculated along with the energy of the structure containing cations of average charge (+4). The resulting energies are plotted as a histogram (Fig. 1). The calculated energies range from $-9,758$ to $-8,421$ kcalorie mol^{-1} with an average value of $-9,356$ kcalorie mol^{-1} which is greater than the energy, $-9,201$ kcalorie mol^{-1} , for the average charge structure. The largest energy is for the structure of columbite.

Figure 1 shows that many possible arrangements of cations have energies not very different from that for the arrangement in columbite. For example, there are 84 cation arrangements (17%) within 158 kcalorie mol^{-1} of the columbite structure. The spread in energies is probably much less than that shown in Fig. 1 because the geometry of the crystal structure used for the calculations and particularly the sizes of the oxygen polyhedra coordinating the cations are optimum only for the cation distribution in columbite. If the crystal structure of each of the 495 cation arrangements could be optimised, the energy of each would probably increase, the net effect being to compress the histogram towards the position of the columbite structure.

Assuming that the value of $-9,201$ kcalorie mol^{-1} is the true energy of the disordered structure, the question "why does one find disordered crystals in nature when the columbite structure is much more stable?" should be recast in the light of Fig. 1 to "why does one find disordered crystals in nature when hundreds of different ordered arrangements of cations are more stable?" The only reasonable answer is that $-9,201$ kcalorie mol^{-1} is not the correct energy of the disordered structure. Therefore, the method of calculating the energy of the disordered structure is in error and one cannot in general determine whether a particular structure is liable or not to cation disorder by comparing average cation charge energies with ordered charge energies.

I propose that the way to make such a judgment is to calculate the electrostatic energy for all possible combinations of cations as done here for columbite. If there are ordered cation

arrangements with energies close to that of the normal ordered structure, then one would expect that partial or complete disorder of the cations would occur because the existence of other cation distributions with not very different energies indicates that the ions can occupy different sites with relatively little penalty in terms of potential energy. But if there is a large energy gap between the normal structure and other possible ordered distributions, one would not expect to find structures with cation disorder. Figure 1 shows that columbite falls into the first category and so disorder is not surprising. Exactly what energy gap is needed for a structure to have only an ordered arrangement of cations is not known but estimates could be obtained by study of appropriate crystal structures as done here for columbite.

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Radiocarbon dates for the Pennine Mesolithic

WE present here a series of ten new radiocarbon dates obtained from charcoal samples collected (1) from sites around Marsden, Yorkshire (Table 1), and formerly stored in the Tolson Memorial Museum, Huddersfield, and (2) from samples collected on upland Pennine sites during the past two years. A single new date from Marsh Benham, Berkshire, is also presented. This short series of dates has some bearing on the relative ages of what Buckley¹ termed the 'broad blade' and 'narrow blade' industries.

The radiocarbon dating of Pennine Mesolithic sites poses several problems. The sizes of the sites vary considerably and there are indications that some may have been occupied for perhaps only a single season, whereas others show signs of lengthy occupation or of having been revisited several times. Datable materials are, however, scarce or absent in all cases, no doubt because of the exposed nature of the upland sites and the constant erosion by high winds and driving rain at these altitudes. The time which elapsed between the abandonment of the sites and their protection by peat formation is unknown, but it was sufficient for most of the datable artefacts to have been destroyed. In the few instances in which charcoal or partially burnt wood has survived in hearths, there is a possibility that contamination has occurred by mineral particles of calcium carbonate and by the down-soaking of humic solutions from the overlying blanket-bog peat. Though these two sources of contamination would have opposite effects on apparent ages they can be eliminated by successive treatments with dilute solutions of hydrochloric acid and alkali. The latter treatment also dissolves a black shiny bituminous substance which sometimes separates from peat and may be confused with charcoal.

Measurements have been made using proportional gas counters with plastic scintillation anticoincidence shielding within a lead castle. Dates have been calculated using the 'Libby half life' of 5,568 yr for the ¹⁴C isotope. Where

Table 1 Radiocarbon dates from Mesolithic sites

Site name	Laboratory number	Age (b.p.)	Date (b.c.)	Un-certainty ($\pm$)
Lominot III*	Q-1187	9,560	7610	350
Marsh Benham*	Q-1129	9,300	7350	150
Warcock Hill South*	Q-1185	9,210	7260	340
Warcock Hill Site III†	Q-789	8,610	6660	110
Broomhead V†	Q-800	8,570	6620	110
Rishworth Draine†	Q-1166	7,500	5600	210
March Hill II†	Q-1188	6,020	4070	220
March Hill II†	Q-788	5,850	3900	80
Rocher Mill Site II†	Q-1190	5,830	3880	100
Lominot IV†	Q-1189	5,610	3660	120
Dunford Bridge B†	Q-799	5,380	3430	80

* Broad blade site.

† March Hill type site.

‡ Rod dominated type site.

samples were small, the technique of dilution with inert carbon dioxide was used. No attempt has been made to calibrate the figures with the bristlecone pine, dendro-chronology curve.

The 'broad blade' industry, now recognised from some 140 locations on the Pennines, was dominated by the manufacture of simple, obliquely blunted points (often retouched on the leading edge) with rare, elongated isosceles triangles and convex-backed blades with a mean width of 8–10 mm. Mellars⁷ has pointed out the Maglemosian affinities of this group, comparing the content of these upland sites with Deepcar, Thatcham, Colne Valley³ and Broxbourne 102 (ref. 4). Confirmation of this purely intuitive correlation is suggested by both computer cluster analysis and a date of $7,615 \pm 350$ b.c. for Lominot site III, "one of two round emplacements" on the eastern side of a hill excavated in 1924 and 1925. That excavation produced 34 microliths³, end scrapers and awls, 90.9% of the raw material being white flint originating from south-eastern Yorkshire and northern Lincolnshire. Similar material has also been found on other typologically identical sites from both upland and lowland areas⁶. The date agrees, within one standard deviation, with determinations from the Zone V/VI part of the occupation in the swamp-marl at Thatcham Site I/V (ref. 7), (Q-677: $6,830 \pm 200$ b.c.; Q-650: $7,720 \pm 160$ b.c.; and Q-652: $7,550 \pm 160$ b.c.) and with a date of $7,350 \pm 150$ b.c. from a new Maglemosian site, with bone, at Marsh Benham.

The second early site to be dated, Warcock Hill South, differs markedly from the other upland 'broad blade' sites in the shortness and relative width of its microliths, the absence of convex-backed points and points retouched on the leading edge, and in its raw materials. Excavated from "within a space of four square yards" (F. Buckley, private printing, 1924) the industry was based on transparent grey, black and honey coloured flints, identical to those of Star Carr and Flixton Site I (ref. 8) with which industries it bears the closest visual similarity, and with which it links statistically, forming a cluster distinct from that which includes Lominot Site III, Deepcar, Marsh Benham and Thatcham sites I/V–III. Pieces of white flint and chert now housed in the Tolson Museum are clearly of post excavation mixing as both of these raw materials are specifically stated by Buckley (unpublished) to have been absent. The radiocarbon date of $7,260 \pm 340$ b.c. agrees, within the statistical uncertainty, with those from Star Carr (Q-14: $7,607 \pm 210$ b.c.; and C-353: $8,217 \pm 560$ b.c.; and $6,858 \pm 490$ b.c.)^{9,10} and could lie, on the basis of the Scaleby Moss diagram, within Zone V, the V/VI boundary here being dated Q-161: $7,059 \pm 194$ b.c. (ref. 10). Warcock Hill South might thus be the contemporary of Flixton Site I, the occupation of which continued into Zone V¹¹.

Sites of Buckley's 'narrow blade' type break down into three distinct groups, each distinguished both typologically and by the presence and proportions of the raw materials used. The largest group, which we here term the 'March Hill' type industry, is dominated by small scalene triangles, normally less than 5 mm wide, and is known from both upland and lowland locations. These lie on the Pennine and Cleveland moors and also occur in northern Lincolnshire and along the Durham^{12,13} and Cumberland¹⁴ coasts. The second group is dominated by straight rod-like microliths blunted along one or two sides. These are known only from high ground on the Pennines and Clevelands. The third group, with many small thomboids, is known in northern England only from two sites, both undated: White Hill North and Red Rafter on the southern Pennines. The first two groups are confined to northern England, and all three lack the inversely retouched leaf and hollow based (Horsham) points characteristic of the sites in south-eastern England.

Radiocarbon dates for the 'March Hill' type industry cover some three millennia. The earliest, $6,660 \pm 110$ b.c., from Warcock Hill Site III, comes from measurements on charcoal from three 'cooking pits' excavated by Buckley and connected with a circular concentration of chert chippings including only a small number of triangular microliths. The date is identical to one from the Broomhead V Site ($6,620 \pm 110$ b.c.)^{15,16} and together these represent the earliest known dates for the late Mesolithic type industries in Britain, agreeing closely with the earliest appearance of small scalene triangles in mainland Europe. That the later dates are correct and not too young is hinted at by the presence of a 'March Hill' type industry in terrestrial deposits on top of the early postglacial raised beach at Eskmeals, Cumberland¹⁴, which accumulated, on the evidence from Silverdale Moss, at about 4,640 bc (Q-260: $6,590 \pm 144$ b.p.)¹⁷. Furthermore, the consistency of the two dates from March Hill Site II, measured on charcoal from either the same, or a pair of cooking pits (Buckley's notebooks are ambiguous) suggests that the dates are correct.

Some charcoal was excavated from a hearth (Rocher Moss South 2), in an eastwards extension of Rocher Moss South 1 (ref. 18). The site also yielded 35 straight, rod-like microliths, but there were no scalene triangles of the March Hill type. This charcoal gave a date of $3,880 \pm 100$ b.c. (Q-1190), which would, along with a date associated with a similar but smaller industry at Dunford Bridge Site B ($3,430 \pm 80$ b.c.; Q-799)^{15,16} suggest a date late in the Mesolithic for 'Rod microlithic' industries of this type.

No comment has so far been made of a site at Stump Cross, Grassington¹⁹ where in 1955 24 flint artefacts were found with charred wood, stratified in mud in a rock cleft. Of five pollen samples associated with the flint, four were representative of the classical pollen zone VIIa which reached 30 cm above it and one represented the early part of Zone VIIa and the Boreal-Atlantic, VIIa/VI transition, 5 cm below it. Measurements of the wood yielded a date (Q-141) of $6,500 \pm 310$ b.c. (refs 10 and 20), which is about 1,000 yr earlier than the usually accepted date of this boundary. Possibly, ancient wood had been blown or washed into the pool and became incorporated into the mud. The two rod-like microliths could derive from either a 'March Hill' or 'Rod' type of industry. The mention¹⁸, however, of the occurrence of chert as a raw material, which, in fact, never appears in industries of the 'Rod' type, suggests that the few artefacts recovered belong to the former, rather than the latter, group.

This radiocarbon evidence from the Pennines indicates conclusively that simple 'broad blade' microlithic industries identical to those of Thatcham and Star Carr precede 'narrow blade' (sometimes termed 'Sauveterrian') industries with small scalene triangles and rod-like microliths, which appear just before the middle of the seventh millennium

bc and persist probably until the end of the Mesolithic. Identical successions can be demonstrated on the basis of radiocarbon dating, stratigraphy and pollen analysis, for the valleys of the Lea, Colne and Kennet in south-eastern England, and also for North Wales, whereas the Zone VIIa pollen age for a group of small scalene triangles from Cock Heads, Glaisdale (J. Bartlett, personal communication) contrasts with the Zone IV and V attribution of the Star Carr and Flixton sites in the nearby Vale of Pickering.

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Possible mechanisms of corona discharge involved in biogenesis

ELECTRIC discharges are very efficient¹ in synthesising amino acids and other organic compounds in conditions which simulate the atmosphere of the primitive Earth. Consequently, they may have been of considerable importance in the origin of life. Corona emission from pointed objects is probably¹ the most effective form of electric discharge involved in prebiotic synthesis. Clearly, coronae occurring in the vicinity of the oceans would be of particular importance, but even though land surfaces generally possess features of sufficient irregularity to permit corona emission in naturally occurring electric fields of moderate strength, it is not obvious that suitable protuberances exist at the ocean surface.

I present here a brief description of experiments which reveal three processes by which coronae can be produced at or near the ocean surface. Two of these processes—the splashing of raindrops in water and the collision of pairs of raindrops—have already been reported^{2,3} and are mentioned here only in view of their possible relevance to prebiotic synthesis. The third mechanism is concerned with bubble-bursting.

When a drop falls into water and splashes, a jet of liquid is ejected vertically upwards. It has a diameter close

to that of the original drop and may rise several centimetres above the water surface. If such splashing events occur in the presence of an electric field, E , the jet is pulled out further by the field, and when E achieves a critical value, E_c , the tip of the jet becomes pointed and a corona is emitted². The value of E_c decreases as the drop radius, R , increases, and for drops of $R=3$ mm, about the largest size existing in natural rainfall, $E_c \approx 180$ kV m⁻¹.

When a pair of raindrops collide, the shapes which they assume before separating depend on their sizes, the velocity of impact, and the obliquity of the collision. In some recent experiments³ a pair of drops, of radii 2.7 mm and 0.65 mm respectively, were brought into collision with a relative velocity of 5.8 m s⁻¹ (which is close to the value appropriate to raindrops of this size falling at terminal velocity in the lower atmosphere). The collisions occurred in the presence of a vertical electric field and corona discharges could be detected. When the drops underwent glancing collisions a long filament of liquid was drawn out between them as they separated. In these circumstances, with the field parallel to the filament, conditions were particularly conducive to the production of coronae, and values of $E_c \approx 250$ kV m⁻¹ were obtained.

Air forced into the oceans by wave motion or precipitation rises to the surface in the form of bubbles. When a bubble bursts at the surface a jet of liquid rises at high velocity from the bottom of the bubble crater⁴ and achieves a height of several millimetres before ejecting a number of drops. It seems possible that the tip of the jet formed when a bubble bursts would provide an effective site for corona emission. Bubbles have been produced by passing air through a tube with a fine capillary tip immersed in water filling a square plastic vessel 0.25 m wide and 0.05 m deep. The bubbles rose to the surface near the centre of the vessel and burst in the presence of a vertical electric field. A reversible polarity, 0–30 kV voltage supply was used to apply a potential difference between the water surface and a smoothed, circular electrode of diameter 0.18 m horizontally mounted centrally above it. The electrode spacing could be varied but was maintained at 0.04 m for the majority of the experiments. Generally, the water was connected to the high voltage supply, the vessel standing on an insulating block. The upper electrode was effectively earthed through an oscilloscope used to monitor the discharges. In other experiments the voltage supply was connected to the upper electrode, or bubbles were allowed to rise about 0.15 m before reaching the water surface. Precautions were taken to ensure that the corona detected was associated with bubble-bursting and not with some spurious process. With the bubble supply switched on, the field was increased slowly until the oscilloscope indicated the occurrence of a corona, which was generally audible. Having thus established a rough value of E_c , observations were made in more detail for values of E around this estimated threshold.

The experiments conducted so far show that corona discharges can occur when bubbles burst at a water surface in fields down to 260 kV m⁻¹. The measured values of E_c are independent of the polarity of the electric field and of the electrode spacing once it is more than about 0.02 m. The independence from polarity is to be expected, in view of earlier experiments^{2,5} concerned with coronae resulting from other mechanisms of the disruption of a water surface. A more detailed investigation of the variation of E_c with bubble size, the charges transferred during the emissions and the mechanics of bubble-bursting in electric fields, will follow.

The three processes by which corona discharges may occur in the vicinity of the ocean surface—drop splashing, bubble bursting and raindrop collisions—are all determined by the production of points at a liquid surface and are not significantly affected by the purity of the water or the

presence of trace gases in the air: this has been confirmed experimentally, some measurements having been made using seawater. All these processes require the presence of strong fields associated with thunderclouds. A full investigation of the coroneae resulting from raindrop collisions and bubble bursting has yet to be made but it seems likely that the minimum values of E_0 for all three processes are in the region of 200 kV m^{-1} . Such values are known to exist within rainshafts and at the surface of the Earth below thunderstorms. The frequency of occurrence of thunderstorms over the Earth is extremely high—of the order of 100 s^{-1} . As the most generally accepted mechanisms of thundercloud electrification depend entirely on the basic physical properties of water and ice, it is likely that a similar frequency of occurrence existed in the primitive atmosphere of the Earth. Although present meteorological knowledge does not enable an estimation of the relative importance of the three processes of corona discharge, it seems quite probable that one or more of them could have been primarily responsible for biogenesis.

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Heavy metal release from plants into the atmosphere

INDUSTRIAL activity has been assumed to be largely responsible for the heavy metal content of atmospheric particulates¹. We have obtained, however, laboratory evidence that higher plants may naturally contribute to the heavy metal composition of the atmosphere. Experiments with radioisotopes have shown that plants are capable of releasing from their foliage a small fraction of the total amount of several elements originally absorbed by their roots. The mechanism giving rise to the release process is as yet not fully understood but seems to be associated with submicron particles. In this way plants could generate local biogeochemical provinces in the atmosphere and reflect underlying geochemical anomalies. Moreover, this

process could also be responsible for the general enrichment of certain heavy metals in the atmosphere relative to the soil and may partly explain the surprising chemical uniformity of atmospheric particulates¹.

The ability of plants to absorb and translocate heavy metals is established². It is also very likely that plants are capable of generating particulate matter in the atmosphere. Went³ was able to show a very strong correlation between airborne submicron particles and density of vegetation. He suggested that these aerosols were derived from condensation of volatile organic material released from the leaves. On the other hand there is also evidence that fine wax particles can be readily lost from the surface of many plants⁴ and that these too could serve as candidates for the formation of particles.

The aim of the experiments reported here was to explore the possibility that heavy metals may be dispersed into the atmosphere by way of a mechanism involving particulate loss from leaves. We report results mainly obtained using radioactive Zn, but several other elements including Pb and Cu have also been studied. Small pea plants (*Pisum sativum*) were normally used, although broad bean (*Vicia faba*) and pine tree seedlings (*Pinus sylvestris*) have also been used. Plants were grown in liquid culture⁵ containing ⁶⁵Zn (Radiochemical Centre, Amersham, as ZnCl₂; specific activity 0.9 mCi mg^{-1}). When the plants were fully labelled with the radioisotope they were transferred to perspex chambers consisting of foliage and root compartments divided by split diaphragms. The roots were sealed off from the upper chamber using Silastic 738RTV placed around that part of the stem passing through the aperture in the diaphragm. Clean dry air was passed through the chambers at known flow rates and the outgoing air monitored for radioactivity.

Preliminary experiments indicated that radioactive Zn was being released from the plants into the air stream passing through the chamber. Controls were conducted on plants after removing their foliage by cutting at a point on the stem just above the sealed aperture. In these conditions no radioactivity was detected in the outgoing air stream. Studies with a Casella cascade impactor demonstrated that the main Zn-carrying components were associated with submicron particles. For this reason in most experiments the outgoing air was passed through first a Millipore filter ($0.22 \mu\text{m}$ pore size) and then a cold finger trap (solid CO₂ and methylated spirit). Radioactivity on the filter and in the frozen water vapour in the cold finger trap (after drying down on planchets) was assayed using a Nuclear Chicago gas flow counter. In most of our experiments the radioactivity given off from the plants was collected in the cold finger condensates and not on the membrane filters. Although the cold finger would tend to trap volatile

Table 1 Release of Zn from leaves of *Pisum sativum*

External concentration (p.p.m.)	Condition	Average Zn level in leaves (p.p.m. fresh weight)	Zn release (pg h ⁻¹ cm ⁻²)	Water transpired (ng h ⁻¹ cm ⁻²)	Zn/water (×10 ⁻¹²)
0.001	Light	7×10^{-4}	0.08	10.0	8.0
	Dark		0.06	6.6	9.1
0.01	Light	5×10^{-2}	0.72	11.6	62
	Dark		0.38	9.0	42
0.1	Light	0.1	4.50	7.0	643
	Dark		2.26	5.6	404
1.0	Light	0.9	21.00	10.6	1,980
	Dark		4.50	8.6	523

Plants were grown in liquid culture medium containing $0.9 \text{ mCi } ^{65}\text{Zn}$ per mg Zn until fully labelled. Illumination was by white light from an incandescent source at an intensity of 100 W m^{-2} . Temperature $25 \pm 2^\circ\text{C}$. Air in foliage chamber was exchanged every minute. ⁶⁵Zn released from the plants was detected only in the cold finger trap (see text) and no radioactivity was found in outlet air for the control experiments. Rates of Zn release are expressed in terms of leaf surface area and computed from 4 h sampling times.

organic compounds released from the plant it also acts as a very efficient trap for submicron particles as shown by studies with a condensation nuclei counter. The trapping of particles by this method almost certainly results from diffusiophoresis⁶ (the Facy effect⁷) brought about by the development of partial pressure gradients associated with water condensation.

Table 1 gives the results of an experiment with pea plants exposed to external Zn levels in the range 0.001–1.0 p.p.m. In this experiment the amount of Zn released increased with increasing external levels of this element and was enhanced by illumination. In general the clearest evidence of Zn release was usually seen under growing conditions and required the plants to be fully labelled with radiotracer. The quantity of Zn given off, however, was variable and on several occasions only a small amount of the element was released even when the plants were grown at relatively high Zn levels. Although Zn release occurred

agent. Of all the metals we have investigated (Pb, Cu, Hg and Mn) all can be released to varying degrees from the leaves by the above petroleum ether or water treatment.

Evidence that Aitken-sized particles are released from plants, under the experimental conditions used have come from electron microscopy studies. A thermal precipitator with a reciprocating head was connected to the outlet of the chamber and any particles released from the plant which passed through the Millipore filter were deposited on a collector grid which had been covered with formvar film and coated with a layer of carbon. Electron microscopy indicated that needle-like particles up to 2,000 Å long and 300 Å wide had been given off from pea plants.

As yet it is not clear whether these particles are associated with the heavy metal release but they do resemble the wax rodlets which occur on many leaf surfaces including the leaves of peas. The occurrence of these small wax crystalline rodlets on the leaf surface is variable

Table 2 Release of ⁶⁵Zn from *Vicia faba* growing in unlabelled and labelled solutions after preloading with ⁶⁵Zn

External Zn concentration (p.p.m.)	Average Zn level in leaves (p.p.m. fresh weight)	Amount of Zn and water released from prelabelled plants in unlabelled solution over 6 h								Amount of Zn and water released from plants when reintroduced to labelled solution	
		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
		Zn	Water	Zn	Water	Zn	Water	Zn	Water	Zn	Water
1.0	1.45	0.96	13.6	0.40	18.4	0	17.2	0	17.6	4.8	12.0
10.0	22.5	10.8	19.2	7.0	24.4	2.24	27.2	1.72	27.2	20.4	15.6

Samples 1–4 were obtained with plants fully labelled with ⁶⁵Zn but having their roots in an inactive culture medium. Sample 5 was obtained after reintroducing the roots to a medium containing ⁶⁵Zn. Daily samples were collected over 6 h so that there was an 18 h gap between sampling. In the case of sample 5 the 18 h period was used to preincubate the plants in radioactive solutions. Sampling was carried out under illumination conditions and in between sampling the plants were exposed to the atmosphere and taken through a day–night cycle. Except for a change in specific activity to 0.09 mCi per mg Zn for the 10 p.p.m. treatment the conditions were the same as for Table 1. Zn and water lost from the plants over the 6 h are expressed in $\mu\text{g cm}^{-2}$ and mg cm^{-2} respectively.

simultaneously with water loss from the plants there was no obvious quantitative association. There was, however, a requirement that the plants were continuously fed with ⁶⁵Zn. Table 2 shows that when a fully labelled plant was placed in a culture solution not containing ⁶⁵Zn, there was a decrease in the ⁶⁵Zn released even though there would have been little or no change in ⁶⁵Zn level in the leaves. Replacement of the plant in the radioactive solution re-established the release process. The release mechanism seems to involve a small Zn pool in the leaf, and the remaining Zn in the leaf is not easily exchanged into this pool. Essentially similar results have been obtained with Cu and Pb but the experiments were more difficult, partly because of variability in the effect and partly because of the relatively low levels of these elements in the leaves. For example, with Pb, levels in the leaves and the outgoing air were reduced by 10 to 50 times of that recorded with Zn when the external levels of these elements were the same (for example, 1 p.p.m.).

The above experiments suggest that the release of ⁶⁵Zn is associated with submicron particles in the Aitken range. A possible source for these could be small particles of epicuticular wax which occur on the leaf surface and which can easily be removed by certain stress conditions such as leaf expansion⁸. Moorby and Squire⁹ tentatively suggested that the loss of these waxes may be responsible for the disappearance of ⁸⁹Sr from leaves which had been surface-contaminated with radioisotope, an explanation also adopted by others¹⁰. We have checked to see if the ⁶⁵Zn absorbed by the roots of our plants is translocated to the vicinity of the leaf surface. Both mild wax extraction procedures¹¹ and leaching experiments¹² indicate that ⁶⁵Zn was present near the leaf surface. Approximately 0.22 p.p.m. Zn was detected in a crude wax extraction with petroleum ether from leaves collected from pea plants grown in 1 p.p.m. Zn and under the same conditions about 50 $\mu\text{g Zn cm}^{-2}$ leaf surface could be leached with distilled water at pH 6.0, containing 0.1% Tween 20 as a wetting

depending on many factors⁴ and could account for the variability observed with the metal release. An alternative explanation for the release could be the production of airborne salt crystals generated by diffusiophoresis associated with rapid transpiration⁸. At this stage we have no positive evidence for either process but the quantitative relationship between heavy metal released and water transpired was highly variable.

Although the rate of metal released is small compared with the total metal content of the leaf it is possible that the release mechanism could give rise to anomalous levels of certain elements in the atmosphere. With a soil water level of Zn at 1 p.p.m. our experiments indicate that the rate of loss of Zn from leaf surfaces can be about 20 $\mu\text{g h}^{-1} \text{cm}^{-2}$. Under these conditions 1 cm^2 leaf surface would contain in the region of 6 ng Zn. Accepting an average leaf area index of 5 (ref. 13) then the rate of Zn release from vegetation to the atmosphere would be 1.0 $\mu\text{g h}^{-1} \text{m}^{-2}$. Such release could, together with human activities, give rise to general heavy metal loadings in the atmosphere. Moreover, our results support those of Curtin *et al.*¹⁴, who reported the release of metals, including Zn, from conifers growing on mineralised soils. Whether the metal-rich particles would normally remain in the Aitken size range in the atmosphere is not yet known but aggregation under the influence of sunlight³ and cohesion with larger particulates may well occur.

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Conditioned bradycardia in the sea lion *Zalophus californianus*

HEART rate is usually reduced when aquatic mammals dive. The degree of this reduction, known as bradycardia, is remarkable¹⁻³, and immersion in water has been regarded as important in its initiation⁴. The heart of the phocid seal beats slower in forced or restrained dives than during trained or voluntary dives^{5,6}, and bradycardia occurs during respiratory pauses in air as well as in water⁷⁻⁹. Central nervous system (CNS) control of bradycardia has been suggested^{5,8,9}. We have investigated whether bradycardia can be conditioned in the California sea lion *Zalophus californianus* since cardiovascular responses can be conditioned in man and many other animals¹⁰.

Radiotelemetry devices were surgically implanted into two sea lions under the external thin muscle layer just posterior to the right axilla. One sensing electrode was on the transmitter package with a second on a lead extending across the thorax about 20 cm toward the ventral midline. The telemetry devices contained a magnetic switch by which the transmitter could be turned off between experiments. The transmitters were still in place and functional 18 months after the operations. Frequent physical and haematological examinations revealed no damage to the animals.

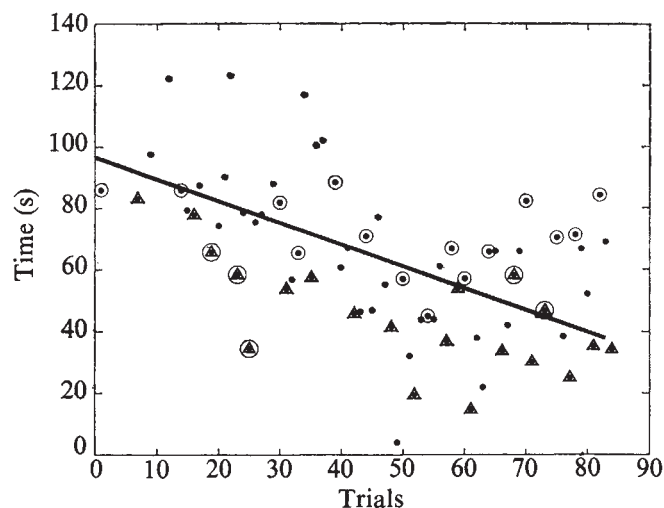


Fig. 1 Each point represents the time required for the sea lion's heart rate to reach 25 beats min^{-1} (as gauged by the two intervals between three successive QRS complexes). Of the 90 3-5 minute sessions of breath holding, 71 were plotted. Nineteen apnoeic periods were discarded because the heart rate remained above 25 beats min^{-1} , ECG data was incomplete because of radio interference, gross movement of the animal, or other equipment related technical problems. The regression line was fitted to these points: circle with dot, first period of apnoea in each training session; triangle with dot, shortest latency, that is the period of apnoea during each training session when the shortest latency between onset of apnoea and achievement of the 25 beat min^{-1} rate was achieved; circle with triangle, first and shortest same, that is, the first period of apnoea in a training session also showed the shortest latency.

After a 2 week recovery period after the operation, one sea lion was conditioned to hold its breath in air (that is, the animal's body was out of the water and dry). The trainer would place his hand near the floor and give the command "down". The sea lion would respond by placing its snout against the trainer's hand and holding its breath until a bridging signal (a police whistle) was sounded. In this way 90 sessions of breath holding of 3-5 min duration were recorded from 20 training sessions during about 2 months. In initial trials bradycardia was slow to develop, and about 90 s were required to reach a basal rate of 25-40 beats min^{-1} from a mean before breath holding of 120 beats min^{-1} . As Fig. 1 shows, the time required to reach a basal rate of 25 beats min^{-1} became shorter with successive trials.

The other sea lion, which had no previous training, was conditioned to reduce its heart rate in response to an acoustic command signal. Ordinarily a sea lion's heart beat cycles with respiration⁷⁻⁹, increasing to 100-140 beats min^{-1} during breaths and decreasing to about 60-90 beats min^{-1} between breaths. These normal episodes of bradycardia were reinforced during initial training. When a criterion of three successive beats at the slowed rate was reached, a bridging signal was sounded and a reward of fish was given. At first the sea lion was required to reduce the rate to 80 or 90 beats min^{-1} . This requirement was gradually reduced a few beats at a time until a heart rate of 10 beats min^{-1} was achieved within 20 s after the bradycardia command was sounded.

The sequence of events was as follows: The sea lion entered a cage. The transmitter was turned on by passing a small magnet by the animal's right axilla. The telemetry signal was received on an f.m. radio, decoded and recorded. The audio output of the radio (a 'beep' for each heartbeat) could be heard by the trainer and the animal. The electrocardiogram (ECG) and command signals were recorded continuously on a Grass polygraph (Model 78). The trainer pushed a button to present the bradycardia command signal. When the heart rate reached the previously determined rate within the prescribed time, the trainer sounded the bridging stimulus, then gave the animal a fish through an opening in its cage. Recordings from one such session are shown in Fig. 2a.

To compare conditioned bradycardia with immersion bradycardia, we decided to repeat Elsner's⁵ experiments. The same sea lion was then trained, using another acoustic command signal, to immerse its head in a pail of water. The results were very similar to those of Elsner⁵. Immersion was followed by bradycardia (25-40 beats min^{-1}) within a few seconds (Fig. 2b). Immersion bradycardia, however, was not as pronounced as that achieved in the previous heart rate conditioning trials (Fig. 2).

Bradycardia that resulted from simple apnoea was much slower occurring than that resulting from immersion or conditioning. In the case of the first sea lion, which was required to hold its breath for a relatively long period (3-5 min), bradycardia developed more rapidly with succeeding trials. This suggests at least two possibilities. The animal may have learned that more prompt bradycardia made breath holding easier, but learning could not be demonstrated by analysis of data from this experiment. The second possibility is that the sea lion's bradycardia became more prompt as a result of physiological adjustment with repetition (that is, analogous to physical training of an athlete).

The second sea lion definitely learned some response that caused a rapid and profound reduction in heart rate. Certainly, breath holding was involved, but the extent of bradycardia achieved during our conditioning experiments was considerably greater than that during similar periods of apnoea or water immersion. Our findings suggest that the sea lion is capable of some control of its heart rate. Such control can be conditioned directly or may come about

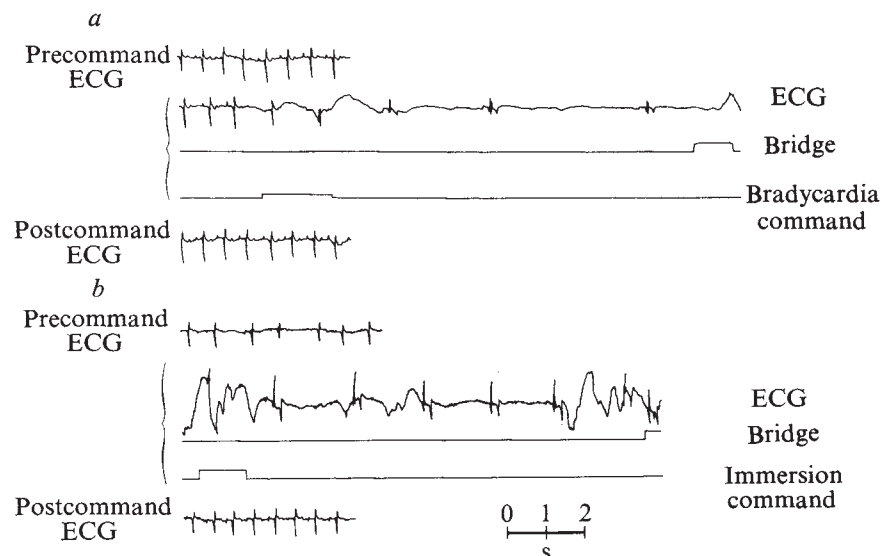


Fig. 2 ECG of the second sea lion. The three different sound stimuli used to signal the animal were indicated on the recordings. *a*, The animal was in a cage in air and dry. The bridge was used to signal correct performance and to indicate to the animal that a fish reward would soon be given. The bradycardia command was meant to signal the animal to reduce its heart rate. *b*, The immersion command signalled the animal to immerse its head in a pail of water. The bridge again signalled correct response after which the sea lion could lift its head from the water and receive a fish.

through some incidental body adjustment of which we are unaware at present (such as, Val Salva's manoeuvre or contraction of respiratory muscles).

The California sea lion has proved to be a tractable laboratory animal that is readily trained to cooperate in experiments. The degree to which it can alter such autonomic processes as bradycardia and peripheral vasoconstriction^{1,2,5} may make it an excellent animal for certain studies involving the CNS control of these responses.

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Precocious sexual parasitism in the deep sea ceratioid anglerfish, *Cryptopsaras couesi* Gill

THE eleven families and nearly one hundred species of ceratioid anglerfish are distributed throughout the world's oceans below a depth of 500 m. The Ceratiidae, with two monotypic genera, *Cerantias* Kröyer and *Cryptopsaras* Gill, is one of four ceratioid families whose members exhibit a peculiar and unique mode of reproduction in which dwarfed males become permanently and parasitically attached to the body of a relatively gigantic female. Males of this family have large, forwardly directed eyes, apparently relying entirely on vision for their search and identification of a conspecific female. As in other

ceratioid males, they are also equipped with a set of pincher-like denticles at the tips of their jaws for grasping and holding fast to a mate. Attachment is followed by fusion of epidermal tissues, and eventually by a uniting of the circulatory systems, so that the male, whose single function is to produce sperm, becomes dependent on the female for blood-transported nutrient, and the female becomes a kind of self-fertilising hermaphroditic host. Since its discovery 50 years ago, the story of sexual parasitism in ceratioid anglerfish has become a part of everyday scientific knowledge, yet no thoroughly satisfactory analysis of the known facts concerning this remarkable reproductive strategy has been made, in spite of the elegant work of Bertelsen¹. This report describes sexual parasitism in surprisingly young females of *C. couesi*. Contrary to previous thought, it is now evident that parasitic attachment can take place at an extremely early age immediately following metamorphosis.

It has long been assumed that before acquiring a parasitic male, female ceratioids must mature to an adult stage, that in some forms (especially ceratiids) is of considerable size²⁻⁴. The smallest known *Cerantias holboelli* with an attached male is 460 mm long⁵ (all fish lengths are measured from the snout to the base of the caudal fin). Before this report, the smallest known, sexually parasitised *C. couesi* female was 176 mm long⁶. In recent years, however, two considerably smaller

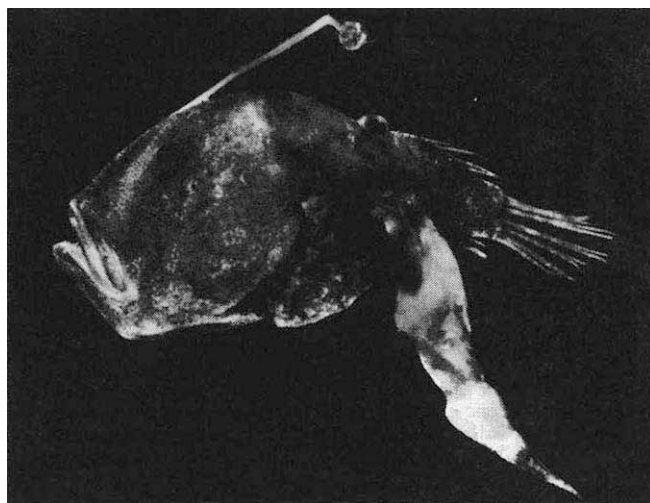


Fig. 1 *C. couesi* female with parasitically attached male, 15.5 and 9.8 mm long (measured from snout to base of caudal fin), respectively (US National Museum, Washington, DC). $\times 3.75$.

C. couesi females with attached males have been discovered. The larger of these is a 77 mm female with a 15 mm male well fused by upper and lower jaws to her belly just to the right of the mid-line (Institute of Oceanographic Sciences, Surrey, UK). Both members of this pair are fully metamorphosed, having darkly pigmented spinulose skin. The testes are large, occupying well over half the volume of the coelomic cavity. In contrast, the paired ovaries are not well-developed, but similar in size to those of non-parasitised females of a similar length.

The second sexually parasitised female *C. couesi* to be described here (Fig. 1; US National Museum, Washington, DC), is remarkable for her small size. At 15.5 mm she is by far the smallest known ceratioid with an attached male. The 9.8 mm male, fused by both upper and lower jaws to the left side of the female just behind the opercular opening, is the smallest known parasitic male of this species. Metamorphosis,

Table 1 Fish lengths and state of gonads for all known parasitically associated females and males of *C. couesi*

Authority	Length*		Gonads	
	Females	Males	Ovaries	Testes
Penrith ¹²	356	73.0	?	Fill body cavity
Penrith ¹²	322	41.0	?	?
Barbour ¹³	290	12.0	Large†	?
Abe and Nakamura ¹⁴	276	12.0	Large†	?
Fast ¹⁶	213	2(27.0–28.0)	Large†	?
Shoemaker ⁶	176	3(16.0–37.0)	Large†	?
Unpublished§	173	35.0	Large†	Large‡
Unpublished	77.0	15.0	Small	Large‡
Unpublished¶	15.5	9.8	Small	Large‡

*Measured (mm) from snout to base of caudal fin.

†Contain eggs visible to the naked eye, some as large as 0.49 mm in diameter.

‡Length greater than 30% of fish length.

§Los Angeles County Museum of Natural History.

||Institute of Oceanographic Sciences, Surrey, UK.

¶US National Museum, Washington, DC.

however, is complete in both sexes. The spinulose skin of the female is darkly pigmented; the ovaries are minute. The skin of the male is spinulose, in contrast to free-living males of this genus⁷. Although the testes are considerably larger than those of free-living *C. couesi* males, its small size, lightly pigmented skin, and well-developed eyes, characteristic of free-living males of this species, indicate the youth of this attached male⁸. Bertelsen⁹ argued that *C. couesi* females take at least 6 months to reach a length of 15–20 mm, and that males of this species have the shortest free-living stage of any ceratioid examined, probably lasting in most cases less than 6 months. If it can be assumed that the parasitic relationship between these two has not impaired normal growth rates, then the members of this attached pair are both quite young, perhaps 6 months and certainly less than 12 months old. As tissue fusion between the two is extensive, attachment probably occurred some considerable time before capture when both partners were at an earlier stage of development than indicated by their present size and condition.

Members of the family Ceratiidae are among the most commonly collected ceratioids. *C. couesi* is now represented in collections by well over 200 metamorphosed females and approximately 75 free-living males. In all of this material, only nine examples of sexually parasitised females are known to the author (Table 1). Examination of the size distribution of these nine females with attached males throughout the bulk of the known material of *C. couesi* (Fig. 2), shows a statistically significant progression of increase in incidence of parasitism with increase in size ($P < 0.001$, by χ^2). Only 0.7% of the 143 females 75 mm and smaller are parasitised by a male. Of the 158 females 150 mm or less, only 1.3% are parasitised, whereas 50% of the 14 females greater than 150 mm are parasitised.

It seems that a female *C. couesi* is able to elicit a search response in a conspecific male, as well as provide cues for specific identification by that male, at a surprisingly early age. Clearly, however, such early attachment is rare.

Gonadal development and sexual maturity of both males and females seems to be dependent on their mutual presence in an obligatory, sexual parasitic association. At least four of the nine known attached males of *C. couesi* have well-developed testes, yet no free-living male with large testes has ever been discovered. Similarly, gravid females are known, but none without an attached male (Table 1). Of the nine known parasitised females of *C. couesi*, most have ovaries with eggs in various stages of development (Table 1). The two

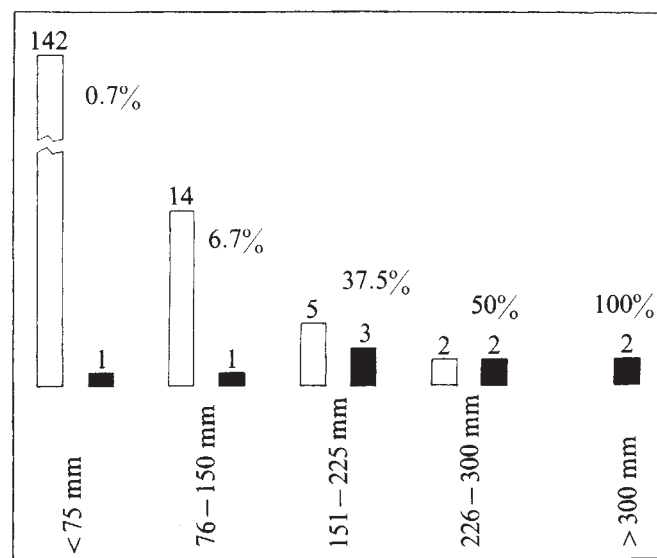


Fig. 2 Distribution by length (measured from snout to base of caudal fin) of nine known sexually parasitised females throughout the bulk of known material of *C. couesi*, showing a statistically significant progression of increase in incidence of parasitism with increase in female size (observed values are significantly different from the expected by χ^2 , $P < 0.001$). Material is divided into five size classes of 75 mm each. White bars indicate number (above column) of non-parasitised and parasitised females, respectively. Percentage of parasitised females is indicated for each size class.

smallest examples, however, are sexually immature, yet their attached males have well developed testes. Had these two small pairs survived the nets of biological oceanographers, the male probably would have remained in a state of sexual readiness for some considerable time until the female reached adult size. On the other hand, perhaps the presence of the male would have led to an early maturation of the female. If so, what have so far been called adolescent females are in actuality 'potential' adults only waiting for the parasitic presence of a male to stimulate ovarian development.

That ceratioid anglerfish, alone among vertebrates, have evolved a reproductive strategy of male dwarfism and obligatory sexual parasitism, can be understood when it is realised that population densities of these organisms are low and mobility restricted by a luring mode of energy capture and by an environment that is vast and productively poor. That parasitic attachment can take place at any time during the apparently long life of a ceratioid^{10,11}, even at a stage immediately following metamorphosis, and thereby making every meeting of a conspecific male and female a potentially sexual affair, makes this solution to the seemingly difficult problem of reproduction in the deep sea, even more remarkable.

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Inversion heterozygosity and the origin of XO daughters of *Bpa/+* female mice

THERE has long been an interest in developing mouse stocks carrying chromosome inversions for mutation testing. This interest stems from the fact that heterozygosity for a paracentric inversion, that is, a structural rearrangement in which a chromosome segment that does not include the centromere is rotated through 180°, results in suppression of recombination in the inversion region. This is caused by the failure of recovery of the products of single crossovers in this region, because dicentric chromatids and acentric fragments are produced that either fail to give functional meiotic products or result in the formation of unbalanced zygotes which are generally eliminated. Recombination between the pair of chromosomes concerned is, therefore, confined to any crossovers in the non-inverted regions and to the rare two-strand double crossovers which may occur within the inversion region if it is a long one. This property of inversion heterozygotes enables the recovery, in the progeny, of the structurally normal homologue largely intact and any recessive mutation located on it can be readily detected if suitable genetically marked stocks are used. In the simplest case, when the mutation is a recessive lethal and the inversion is located in the X chromosome, detection is either by the absence of half the males, or if the inversion itself is marked by a recessive lethal (as in the C1B method devised by Muller for *Drosophila melanogaster*), by the absence of all the males.

Roderick¹ has made a systematic search for autosomal inversions in the mouse but his method does not recover inversions in the X chromosome. An X chromosome inversion, however, besides being simpler to use, would be far more efficient for mutation testing primarily because detection could be achieved in only two generations rather than the three that would be required using an autosomal inversion. We now report the finding of such an inversion among the descendants of a male that had received a fractionated dose to the spermatogonia, of 12 × 50 rad X rays given at weekly intervals. It was found associated with bare patches (*Bpa*), a mutation which proved to be sex-linked and male lethal². The original *Bpa/+* females produced, among their progeny, considerable numbers of XO daughters all of the rare OX^p type³. This enhanced capacity to produce XO progeny was subsequently shown to be separable from *Bpa* and was termed *Fxo* (ref. 4). The presence of *Fxo* also suppressed crossing over between *Bpa* and *Ta* or *Blo* and it was suggested that a structural mutation of the X chromosome, perhaps an inversion, might be involved⁴.

A long paracentric inversion of the X chromosome has now been demonstrated cytologically in *Bpa Fxo/+* animals

and shown to be absent from *Bpa+/++* animals; therefore the symbol *Fxo* has been withdrawn and replaced by *In(X)1H* (ref. 5) in accordance with the standardised nomenclature for the mouse⁶.

The inversion was first identified cytologically by the presence of characteristic bivalents at diakinesis in oocytes from *Bpa In(X)1H/+* heterozygotes and was confirmed in Giemsa-banded preparations⁷ made from bone marrow. Preliminary observations suggest that it extends from a proximal break point lying just within the dark centric band (A1 of Nesbitt and Franke⁸) to a point distal to band E and that it represents about 85% of the physical X chromosome (Fig. 1).

An average of 30 chiasmata per oocyte has been observed

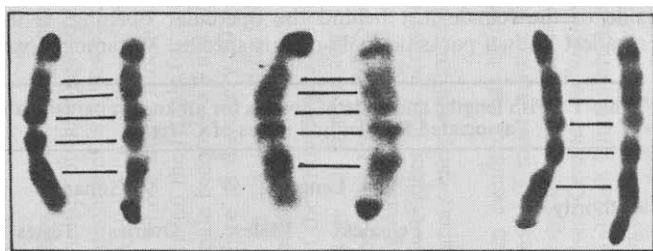


Fig. 1 X chromosomes from three cells heterozygous for the inversion. *In(X)1H* chromosome is on the right of each pair and inverted in relation to its normal homologue. Lines join presumptively homologous bands. Direct bone marrow preparations stained by trypsin-Giemsa method.

at diakinesis in normal, structurally homozygous mice (C. E. Ford and E. P. E., unpublished). If these are partitioned between bivalents in proportion to the lengths of the chromosomes at somatic mitosis⁹, the mean number in the X bivalent would be 1.81. Distributing these in proportion to segment length, the mean number of chiasmata within the inversion region would be approximately 1.54, which would correspond to a genetic length of 77 centiMorgans (cM). This is greater than the total length of the current X chromosome map (75 cM; Fig. 2) and would imply that most, perhaps all, the mapped loci lie within the inversion.

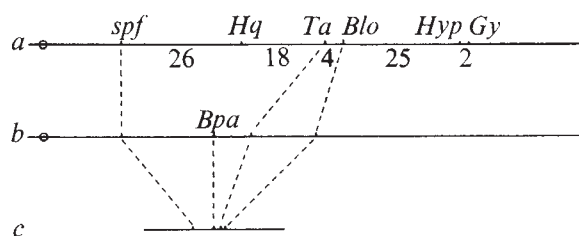


Fig. 2 Map of the X chromosome of the mouse showing the effect on recombination of *In(X)1H*. a, Standard map; b, from *Bpa* non-*In(X)1H* females; c, from *Bpa In(X)1H* females. Units represent percentage crossover.

A minimum estimate of the length of the inversion can be obtained independently from the genetic evidence. Data on the recombination between *Bpa* and the other three loci in the absence of *In(X)1H* (Table 1) indicate that *Bpa* lies between *spf* and *Ta*. Strong crossover suppression had been shown to occur between *Bpa* and *Ta*, and between *Bpa* and *Blo* (ref. 4); it has now also been demonstrated between *Bpa* and *spf* as well (Table 1 and Fig. 2), that is, crossover suppression occurs on both sides of *Bpa*. Also recombination between *Bpa* and either of the markers *Ta* or *spf*, leaves *In(X)1H* on the unmarked chromosome (Table 1). Together, this evidence implies that *Bpa* lies within the inversion, and therefore to separate *Bpa* from *In(X)1H* would require a double crossover with one

Table 1 Segregation from: (1) *Bpa*+/*+**Ta*×*Blo* Y*; (2) *Bpa*+/*+**Blo*×*Ta* Y*; and (3) *Bpa*+/*+**spf*×*spf* Y in the presence and absence of In(X)1H

Cross using <i>Bpa</i> In(X)1H chromosome	Female progeny				Male progeny		% Recombination (non- <i>Bpa</i> progeny only)	5% Fiducial limits	
	<i>Bpa</i> † OC†+RC†	non- <i>Bpa</i> OC†	RC†	XO	non- <i>Bpa</i> OC†	RC†			
(1)	54	131	3	50	127	1	1.53	0.42 and 3.91	
(2)	77	111	1	67	88	4	2.45	0.79 and 5.72	
(3)	22	37§	2	23§	39	1	3.80	0.78 and 11.01	
<i>Bpa</i> + Chromosome									
(1)	66	129	12	—	125	11	8.30	s.e. ±1.66	
(2)	51	55	11	—	58	14	18.16	±3.28	
(3)	25	38	15	—	49	12	23.68	±3.98	

*Includes data from ref. 4.

†The crossover of any of the genes on to the same chromosome as *Bpa* is not definitely identifiable, therefore all *Bpa* offspring are combined.

‡OC, old combinants; RC, recombinants.

§Identified by corneal mitoses. A further 15 *spf**spf* or *spf*0 have died unclassified.

||All three recombinants from cross (1) and one from cross (3) have been tested and shown still to carry In(X)1H.

exchange on each side of the *Bpa* locus. The frequency of separation has been estimated by testing *Bpa* daughters of *Bpa* In(X)1H mothers not carrying any other sex-linked gene. Seven of 87 such daughters proved to have lost the inversion ($8.05 \pm 2.92\%$).

If *Blo* does lie outside the inversion, recombinants between *Bpa* and *Blo* in the presence of the inversion would include all *Bpa*-In(X)1H double crossovers plus any crossovers between the distal break point of the inversion and the locus of *Blo*. But the estimated recombination between *Bpa* and *Blo* (2.45%) is less than between *Bpa* and the inversion (8.05%); *Blo* therefore probably lies within the inversion. The same argument can be applied to *Bpa* and *spf* (3.80%), and *spf* more likely than not lies within the inversion. The genetic data are, therefore, consistent with an inversion greater than 48 cM long.

At the stage when the tentative hypothesis for an inversion was first put forward the non-*Bpa* In(X)1H chromosome had not been obtained⁴. Subsequently both +In(X)1H/+ and +In(X)1H/Y animals have been produced. The females as expected, give a similar percentage of XO progeny to their *Bpa* In(X)1H/+ counterparts, but the males do not give XO offspring.

A greatly increased frequency of XO daughters would be anticipated among the progeny of females heterozygous for an X chromosome inversion. The dicentric chromatid resulting from a single chiasma within the inversion region would be expected to lead to a chromatid bridge at anaphase I. Whether this was left on the spindle, broken, or incorporated intact into the secondary oocyte (or polar body) nucleus, its eventual elimination would be inevitable, and although the dicentric itself has not yet been recognised a first rough assessment suggests that the frequency of bivalents with a single chiasma in the inverted segment is about 0.4. Elimination could occur either at meiosis or subsequent to fertilisation. In either case an equal frequency of XO and OY individuals would be expected. Crossing over within the inversion is necessarily excluded in In(X)1H/Y males and no XO offspring would be expected.

On the basis of all this evidence, therefore, it seems certain that we are dealing with a very long sex-linked inversion. The occurrence of double crossing over would reduce its efficiency for mutation testing compared with the Muller-5 stock of *Drosophila* although this may be more than compensated for by the accurate estimates of probability of mutation that would be possible. In any case a technique based on the In(X)1H inversion, already marked by the male-lethal mutation *Bpa*, would be far more efficient than the methods at present in use for estimating the frequency of sex-linked lethals in mice¹⁰.

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Limited potential of circulating haemopoietic stem cells

It has long been known that animals irradiated with a lethal dose may recover if the spleen is shielded during exposure¹, or if normal bone marrow or spleen cells are subsequently injected intravenously². Recovery has been shown to be the result of repopulation of the damaged haemopoietic tissues by stem cells from the donor³. It was later found that cells with at least some of the characteristics of stem cells were present in the blood of normal mice^{4–6}. These characteristics included the formation of spleen colonies and the ability to promote recovery from lethal irradiation, at least in the short term. Some studies⁷ have suggested that circulating stem cells are equivalent to those in bone marrow, while others⁸ have indicated certain differences.

In spite of evidence that at least some stem cells are mobile, other data imply that the actual interchange of stem cells between different bone marrow sites may normally be limited or absent^{9–14}. Calculations¹⁴ show that, at most, only a small proportion of circulating stem cells enter the bone marrow and find haemopoietic clones. A possible explanation for this is that circulating stem cells have a limited capacity for self renewal. This hypothesis has been tested in two experimental systems.

The first approach was to investigate the relative regeneration of spleen colony-forming units (CFU)—stem cells which home to the spleen—in spleens carrying colonies derived from bone marrow or blood. The experimental scheme and results of four experiments are shown in Table 1. In addition, radioiodinated 5-iodo-2-deoxyuridine (IUdR) was injected into mice in two of the experiments to measure DNA synthesis (Table 2).

Table 1 CFU renewal indices (RI) in the spleens of lethally-irradiated CBA male mice injected with bone marrow (BM) or blood cells*

1 Exp.	Primary recipients		Spleen cells transferred to each secondary recipient		6 Mean no. spleen colonies†	Secondary recipients	
	2 Nucleated cells injected ($\times 10^{-6}$)	3 Mean no. spleen colonies†	4 No. of nucleated cells ($\times 10^{-6}$)	5 No. of colony equivalents		7 CFU RI	8 Ratio of RI — BM: Blood
1 BM	0.1	14.3 (7)	1.4	2.5	21.0 (10)	8.4	15.6
Blood‡	2.2	3.3 (7)	4.6	2.3	1.3 (8)	0.5	
2 BM	0.05	9.6(11)	1.4	1.8	8.0 (8)	4.4	>48.5
Blood	4.0	3.0 (5)	2.4	1.5	0.0 (8)	< 0.1	
3 BM	0.05	14.2 (8)	5.0	4.5	14.3 (6)	3.2	18.3
Blood	4.5	14.9 (7)	13.8	9.0	1.6(12)	0.2	
4 BM	0.05	11.9(12)	4.5	4.8	11.3(20)	2.4	9.5
Blood	9.0	21.2 (5)	11.6	14.8	3.7(20)	0.3	
	10-d assay:		1.5	1.6	17.4(19)	11.2	8.6
			16.8	15.9	20.9(20)	1.3	

*Normal BM or blood cells were injected intravenously to lethally-irradiated (900 rad) primary recipients. Eight days later the recipients were killed. Their spleens were divided into two groups; half were fixed for counting of spleen colonies; the remainder were pooled and cell suspensions were prepared in Hanks' solution for intravenous injection to secondary 900 rad-irradiated recipients. These were killed 8 d later, after injection of ^{125}I -IUdR (see Table 2), and their spleen colonies counted. The RI (column 7) was calculated as column 6/column 5 (that is, CFU per colony equivalent injected). Irradiated, non-injected control mice never developed spleen colonies.

†Numbers of mice in parentheses.

‡Heparinised blood was injected whole (experiment 1), or after sedimentation of erythrocytes with Plasmagel (Lab. R. Bellon, Neuilly, France).

The stem cell population derived from blood, assayed as CFU, renewed its numbers far less effectively within 8 d than that derived from bone marrow. The few colonies that did develop in the secondary recipients of blood-derived spleen cells were mostly small, and barely detectable without a hand lens; recipients of marrow-derived spleen cells, on the other hand, developed colonies of normal size. DNA synthesis (and thus cell proliferation) was also relatively slight, although the difference between the groups was less than would have been predicted from the colony counts. Some of the cell proliferation may be attributable to the descendants of injected splenic lymphocytes (originally derived from the primary blood inoculum); terminally differentiating microcolonies of haemopoietic cells may also contribute. The circulating stem cells of mice rendered anaemic with phenylhydrazine have also been shown to have relatively poor powers of self renewal¹⁵.

In experiment 4, the doubling time of CFU between day 8 and day 10 was the same in marrow-derived and blood-derived colonies (20–21 h), which agrees with the data of Gidali *et al.*⁸.

The overall regeneration of haemopoietic populations

derived from blood and bone marrow stem cells was compared directly by means of chromosome markers. Groups of mice were injected with a mixture of syngeneic bone marrow and blood cells distinguishable by the possession of one or two T6 chromosomes and killed at intervals for cytological examination of mitotic cells¹⁶. Although the presence of the markers does not affect the proliferative fitness of a population¹⁷, two experimental groups were set up with reciprocally marked donor cells as a precaution. The cell doses were weighted 100-fold in favour of blood, so as to obtain roughly equal numbers of stem cells initially. The suspensions were tested individually for their CFU content. During the second week after injection, the contribution of blood-derived cells to the mitotic populations in the haemopoietic system dropped sharply. By day 23 they constituted less than 5% of the total donor-derived population (Fig. 1).

These experimental results imply that blood and bone marrow stem cells differ markedly in their self renewal, and in the size of descendant populations which they produce in identical conditions. A possible interpretation of the spleen colony data, taken in isolation, would be that blood CFU

Table 2 Splenic uptake of IUdR in lethally-irradiated recipients of cells from colony-containing spleens*

Exp.	Cell injected to primary recipients	Splenic IUdR uptake (geometric mean) in secondary recipients	
		Total specific uptake/spleen†	Specific uptake per colony-equivalent injected (ratio BM: blood)
2	BM	967 (8)	528
	Blood	182 (8)	121
			(4.4)
3	BM	2,905 (6)	653
	Blood	1,445(12)	160
			(4.1)

*Details of mice are given in Table 1. Three hours before death mice were injected intraperitoneally with 5×10^{-8} mol fluorodeoxyuridine followed after 1 h by $1 \mu\text{Ci } ^{125}\text{I}$ -IUdR (specific activity, 5 mCi mg^{-1} ; Radiochemical Centre, Amersham) as previously described²⁰. After washing out of non-DNA-associated radioactivity, spleens were counted in a Packard crystal scintillation counter. ^{125}I -uptake was expressed as a proportion of the injected activity; counts $\times 10^6$ per injected counts.

†Numbers of mice in parentheses. 'Specific' uptake was that attributable to the donor cell population, after subtraction of the mean uptake in irradiated, non-injected controls.

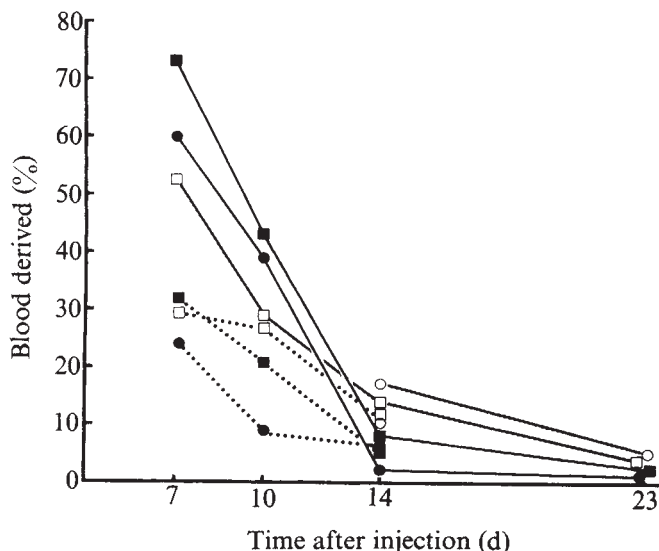


Fig. 1 Identity of mitotic cells in tissues of lethally-irradiated (900 rad) CBA mice injected intravenously with chromosome-marked syngeneic bone marrow and blood cells. Experiment A (---): T6 bone marrow (5×10^4 nucleated cells, 4.0 CFU equivalents)+T6 blood (5×10^6 nucleated cells, 2.5 CFU equivalents) injected. Experiment B (—): T6 bone marrow (5×10^4 nucleated cells, 2.5 CFU equivalents)+T6 blood (5×10^6 nucleated cells, 8.1 CFU equivalents) injected. Mean number of mitoses scored per tissue: bone marrow 58, spleen 91, thymus 100, lymph nodes 77. ●, Bone marrow; ■, spleen; ○, thymus; □, lymph nodes.

(and/or their CFU descendants) have different homing or migratory properties from marrow CFU, without necessarily differing in their rate of growth. The results of the competition experiment (Fig. 1) argue against such an interpretation. Taken together, the data strongly suggest that although blood CFU are able to engender differentiating clones which grow within 1–2 weeks to perhaps a few million cells, they have, on average, little ability to increase their numbers and thus produce larger and more persistent clones. The presence of a minority of relatively efficient stem cells in the blood is suggested by the similar growth rate of blood-derived and marrow-derived CFU between 8 and 10 d following transplantation.

Calculations based on a stochastic model of haemopoiesis¹⁸ have suggested that in conditions of haemopoietic regeneration in the spleen, the average probability of two new CFU resulting from the division of an existing CFU is 0.62–0.64 (refs 19, 20); in these conditions the CFU pool expands. If this probability is similar for bone marrow stem cells under steady-state conditions, a certain surplus of CFU will be produced continually in the bone marrow. Such a surplus may either be eliminated *in situ*, or discharged into the bloodstream. These processes may be random, or they may act selectively on, for example, stem cells with a relatively long mitotic history. The present results show that stem cells in blood are not a random sample of those in bone marrow, but are a selected population of generally low proliferative potential. Serial subculture of diploid cells *in vitro*^{21,22} and serial transplantation of tissues^{23–25} and haemopoietic cells *in vivo*^{3,20,28–29} cannot be continued indefinitely; after a certain number of divisions the cells cease to multiply. By analogy, it is reasonable to speculate that the CFU normally found in blood are victims of clonal senescence, and have been expelled as waste products from the bone marrow.

Evidence for the low proliferative potential of circulating stem cells is derived almost entirely from work on bachelor male mice of the inbred strain CBA and its congenic line CBA-T6, bred and maintained in conventional conditions. It remains to be seen whether this potential is equally low

in other animals and whether it can be enhanced by experimental means.

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Independent regulation of cellular properties in thermosensitive transformation mutants of mouse fibroblasts

CHEMICAL¹ or viral² transformation of fibroblasts in tissue culture results in cells with altered properties (for review see ref. 2). These properties can be broadly subdivided into those associated with changes in the plasma membrane³ and those associated with intracellular regulatory changes². The former changes include the ability to grow in soft agar⁴, the loss of an iodinated surface glycoprotein^{5,6} and an increase in total cell-membrane-associated proteolytic activity⁷ on cell transformation. The chief manifestation of intracellular changes is the failure of transformed fibroblasts to cease growing at a specific point (G_1 or G_0) in the cell cycle⁸ and thus to express the typical increases in protein synthesis, ribosomal RNA synthesis and DNA synthesis upon growth reinitiation⁹. We have now investigated some of these properties in cell mutants of BALB/c-3T3 which show temperature-dependent changes in many properties characteristic of the transformed state in tissue culture. These mutant cells behave as normal mouse fibroblasts in the regulation of their internal cellular machinery at permissive or non-permissive temperatures but show temperature sensitive changes in those properties we have measured which are considered to be associated with membranes.

Both virus and cell mutants which give rise to temperature sensitive (ts) lesions in many properties characteristic of transformed fibroblasts have been reported^{10–12}. Eckhart has

Table 1 Summary of intracellular changes after addition of serum

Cell type	Temperature (°C)	Ratio: newly growing cultures/resting cultures			
		Protein synthesis	rRNA synthesis	DNA synthesis	Cell no.
BALB/c-3T3	32	2.0	2.0	95	1.9
	39	1.8	2.0	86	1.8
ts Cl.1	32	2.0	2.3	35	1.7
	39	2.4	2.0	68	1.9
ts Cl.10	32	2.6	2.3	39	1.7
	39	2.5	1.8	90	2.0
ts Cl.X	32	2.0	1.9	30	1.8
	39	2.0	2.0	55	1.8
Py3T3-Swiss	32	1.1	1.0	1.3	1.1
	39	1.1	1.1	1.4	1.0
SV3T3-BALB/c	32	1.1	1.1	1.0	1.0
	39	1.2	1.0	1.1	1.1

Non-transformed (BALB/c-3T3) mutant (ts Cl.1, ts Cl.10, ts Cl.X) and virally transformed -3T3 (BALB/c-SV3T3, Swiss Py3T3) cells were grown as described previously¹³ in Dulbecco's modified Eagles medium (DEM) containing 10% foetal calf serum at either 32 or 39 °C until they formed a confluent monolayer. The medium was removed and replaced with DEM and 1% foetal calf serum for 3 d. Then the medium in half the cultures was replaced with fresh DEM and 20% serum. Protein synthesis was measured by exposing the cultures to 0.2 $\mu\text{Ci ml}^{-1}$ of (^{14}C -U) amino acid mixture (specific activity 57 mCi per matom) from 4 to 6 h at 39 °C or from 8 to 12 h at 32 °C; RNA synthesis with 1 $\mu\text{Ci ml}^{-1}$ ^3H -uridine at 5×10^{-6} M from 10 until 16 h at 39 °C or from 20 until 32 h at 32 °C. The fraction of cells with ^3H -thymidine radioactively labelled nuclei⁹ was recorded for cells exposed to ^3H -thymidine throughout the DNA synthetic period (from 10 until 30 h at 39 °C and from 20 until 60 h at 32 °C), and the cell numbers were recorded after 48 h at 39 °C and 96 h at 32 °C respectively. The radioactivity incorporated into protein was expressed per mg of cell protein. The radioactivity of ^3H cytoplasmic ribosomal RNA (rRNA) synthesised in the time period per mg of cell protein was corrected for the differential incorporation rates into the trichloroacetic acid-soluble RNA precursor pool as previously described^{9,22}. Previous results for changes in nontransformed fibroblasts³ cytoplasmic rRNA synthesis by this method agreed with those obtained by other authors from direct measurements of intracellular specific activities of the RNA precursors²². Incorporation of ^{14}C amino acids into protein or ^3H -uridine into RNA (18S and 28S) increased linearly with the time of exposure to the radioactive macromolecular precursor. Results are expressed as a ratio of the synthesis or cell number after quiescent cultures were induced to grow with fresh serum to those values before the culture medium was changed. Errors in the ratios are approximately 10–15% for protein, RNA synthesis, and cell numbers, and 20–25% for the radioactively labelled nuclei.

isolated different clones of BALB/c-3T3 cells which grow in agar at 32 °C (W.E., unpublished). Cells from these clones show transformed morphology, ability to grow in agar, higher growth rates and higher saturation densities at 32 °C than at 39 °C, although at low concentrations of serum in the tissue culture medium they exhibit density-dependent inhibition of growth at both 32 °C and 39 °C similar to non-transformed cells but unlike the virally transformed cell lines Py3T3 and SV3T3¹³. In this paper three independent ts mutant clones of BALB/c-3T3 cells (ts Cl.1, ts Cl.10 and ts Cl.X) were tested for temperature-dependent changes in various cell properties. Cells were grown in 10% serum and then arrested in their growth by a reduction in the serum concentration to 1%. On addition of fresh medium and 20% serum to the quiescent mutant cells at either 32 or 39 °C they increased the overall rate of protein synthesis, cytoplasmic RNA synthesis (28S and 18S RNA), initiated DNA synthesis and divided (Table 1) just as their parent cell⁹. The time scale for the increase in macromolecular synthesis and cell division was twice as slow at 32 °C than at 39 °C (Table 1). In particular for both the mutant and BALB/c-3T3 cells at 39 °C DNA synthesis was

initiated at 8–10 h, reached a maximum at 18–20 h and was followed by virtually complete cell division by 40 h, whereas for parent and mutant cells at 32 °C DNA synthesis was initiated at 16–18 h, reached a maximum at 34–36 h and was followed by nearly complete cell division by 80 h. Thus the mutant cells were uniquely arrested in growth in the same position of the cell cycle as their parent BALB/c-3T3 cell (G_1 or G_0) at both temperatures and had not ceased growing at random¹⁴. The fully transformed BALB/c-3T3 cells SV3T3 and Swiss Py3T3 did not show density-dependent inhibition of growth at low serum concentrations, and thus did not appreciably increase rates of macromolecular synthesis and cell division on readdition of 20% serum (Table 1).

Recently the loss of a large, external, transformation-sensitive (LETs) protein has been described after viral or chemical transformation of fibroblasts^{5,6}. This has been demonstrated using lactoperoxidase-catalysed iodination. The mutant clones of BALB/c-3T3 cells were arrested in their growth at confluency (Table 1), then radioactively labelled with ^{125}I *in situ*, and the iodination patterns analysed on SDS-polyacrylamide gels (Fig. 1). The radioactive LETs

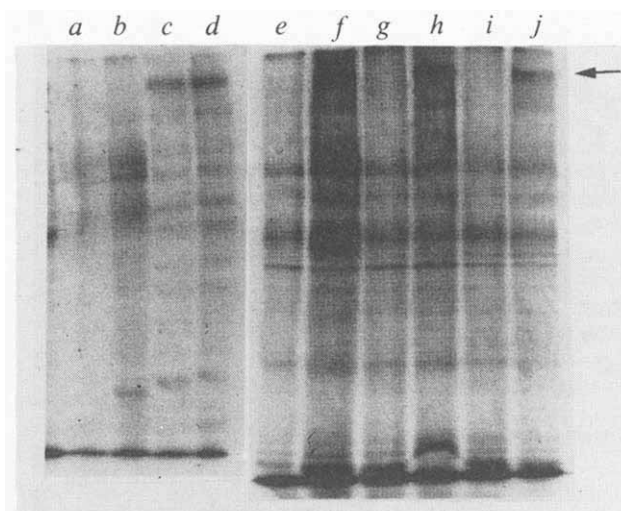


Fig. 1 Autoradiograph of ^{125}I -labelled cells after SDS-polyacrylamide gel electrophoresis. BALB/c-SV3T3 cells grown at: a, 32 °C and b, 39 °C; BALB/c-3T3 at c, 32 °C and d, 39 °C; ts Cl.1 at e, 32 °C and f, 39 °C; ts Cl.10 at g, 32 °C and h, 39 °C; ts Cl.X at i, 32 °C and j, 39 °C. Confluent cultures of cells were iodinated as previously described⁶ directly on the dish. Before iodination, the cells were washed twice at room temperature with phosphate-buffered saline (PBS), pH 7.2 and to each 5-cm dish was added 0.5 ml of PBS containing D-glucose (0.9 mg ml^{-1}) and carrier-free Na^{125}I (400 $\mu\text{Ci ml}^{-1}$). Then 10 μl of an enzyme mixture containing lactoperoxidase (1 mg ml^{-1}) and glucose oxidase (0.1 U ml^{-1}) was added for 10 min at room temperature. The reaction was stopped by the addition of 5 ml of 0.17 M NaI in 0.01 M sodium phosphate buffer, pH 7.2. The cell monolayers were then washed twice with PBS, the cells were scraped off the dish with a rubber policeman and rewashed with 0.1 M Tris-HCl (pH 6.8) containing 15% glycerol. Samples of 10^6 c.p.m. were collected by centrifugation, adjusted to 2% sodium dodecyl sulphate and 0.1 M dithiothreitol, and boiled for 2 min. Polyacrylamide gel electrophoresis²³ was performed in a slab gel at an acrylamide monomer concentration of 7.5%. After electrophoresis, the slab gel was dried in a vacuum and autoradiographed for 4 d using Kodak X-ray film RP/R54 to locate the radioactively labelled components.

glycoprotein of nominal molecular weight 250,000 was observed in the parent cells and the ts cells at 39 °C but had virtually disappeared in the ts cells at 32 °C and the BALB/c-SV3T3 transformed fibroblasts. Several other radioactively-labelled components disappeared when the mutant cells were grown at 32 °C instead of at 39 °C, although whether these reflected changes in plasma membrane composition or differential adsorption of serum components to the cells at the two temperatures is unknown. The same pattern was observed if the mutant cells were grown in 10% serum and radioactively labelled when confluent (not shown).

The cell factor that is involved in tumour-associated fibrinolysis occurs in the postnuclear particulate fraction of Rous sarcoma virus transformed chick embryo fibroblasts, although its exact membranous location has not yet been determined⁷. This arginine-specific protease converts serum plasminogen to its enzymatically active form plasmin. The plasmin in turn can be detected by its capacity to hydrolyse ¹²⁵I-fibrin¹⁵ or the milk protein casein in an agarose overlay assay¹⁶. Cells were grown as before (Table 1) and assayed for the production of the cell factor by the degree of hydrolysis of casein with either 1% monkey serum, 0.5%

permanently at 32 °C in all the tests used, and *vice versa*. After more than 20 passages in cell culture ts Cl.1 cells started growing in agarose at 39 °C and simultaneously the iodinated LETS glycoprotein was not detectable and the proteolytic activity increased from 0 to +2.

Previous reports have indicated that many of the properties characteristic of the transformed state change in a coordinate manner on viral transformation of fibroblasts. But the isolation of stable SV40-transformed 3T3 cells with partially transformed characteristics¹⁷⁻¹⁹ and SV40-transformed rat embryo cells which continuously express the viral T antigen but coordinately vary in anchorage dependence and plasmin activator production²⁰, have demonstrated that SV40-induced transformation of fibroblasts can lead to a variety of stable changes. The dependence of BALB/c-3T3 fibroblasts on anchorage for growth may be directly responsible for their failure to induce tumours in animals²¹. We have now shown in the same temperature-sensitive mutant cell that those properties considered to be associated with the surface membrane, the ability to grow in agarose, the disappearance of the iodinated glycoprotein, the loss of the growth-promoting ability of the fibroblast growth factor¹³ and the increase in total cell membrane proteolytic

Table 2 Proteolytic activity and growth in agarose

Cell type	Temperature (°C)	Degree of casein hydrolysis (scale 0 to 4)			Colony formation (%)
		Monkey serum	Dog serum	Plasminogen	
BALB/c-3T3	32	1	0	0	0.3
	39	0	0	0	0.6
ts Cl.1	32	3	3	2	21
	39	0	0	0	0.9
ts Cl.10	32	3	3	2	28
	39	0	0	0	0.5
ts Cl.X	32	4	3	3	25
	39	0	2	0	6.6
Py3T3-Swiss	32	ND	4	3	41
	39	ND	3	3	40
SV3T3-BALB/c	32	4	4	4	ND
	39	4	4	4	ND

For the measurement of fibrinolytic activity the conversion of serum plasminogen to plasmin by the cell activator⁷ was measured by the plasmin-induced hydrolysis of casein¹⁶. Cells grown at their respective temperatures were plated at 10^5 (Monkey serum) or 2×10^5 (dog serum and plasminogen) per 3.5 cm Petri dish in DEM and 10% foetal calf serum. After 3 h the medium was removed, cell monolayers were washed twice with DEM and then 1 ml of a suspension of 0.6% agarose, 2% autoclaved powdered milk (Marvel) mixed with one of 1% African green monkey serum (Flow Laboratories) or 0.5% dog serum (Gibco) or $30 \mu\text{g ml}^{-1}$ of plasminogen purified from foetal bovine serum (gift of Dr R. Hynes) in DEM was added and allowed to solidify at room temperature. Finally, 1.5 ml of 0.6% agarose in DEM was added, allowed to solidify and the dishes were then incubated at 32 or 39 °C until the casein in the dishes containing SV3T3 cells had completely lysed (approximately 40–50 h at 39 °C and 80–120 h at 32 °C depending on the serum used). The remaining plates were scored on a scale of 0 to +4 (complete hydrolysis) according to the proportion of casein removed. Similar results were obtained with 0.25%, 1% and 2% dog serum, but with 10% dog serum there was virtually no differences for the ts cells at 32 °C or 39 °C. Petri dishes containing no transformed cells but with serum or plasminogen, or containing transformed cells but no serum or plasminogen gave no detectable casein hydrolysis, while 0.005% trypsin (w/v) completely hydrolysed the casein within 48 h at either 32 °C or 39 °C. Fibrinolytic assays¹⁵ using the same cells and sera failed to yield definite differences in the rate of proteolytic hydrolysis of ¹²⁵I-labelled fibrin (not shown). The ability of different cells to grow in agarose was determined by plating 10^4 or 10^5 cells in liquid medium containing 10% foetal calf serum in 5 cm Petri dishes. Cells were overlaid with a suspension of 0.33% agarose and 10% foetal calf serum in DEM, incubated at 32 or 39 °C, and the colonies counted after 2, 3 and 4 weeks. The percentage (%) of cells which grow in agarose is shown. The figures represent the average of 3 separate experiments (8 Petri dishes per experiment) and 2 microscopic fields of 200 cells were counted per Petri dish. The ts cells formed appreciably smaller colonies at 32 °C than the Py3T3 cells. ND, not determined.

dog serum, or purified bovine plasminogen (Table 2). Calf serum contains an inhibitor of the plasmin assay¹⁵ and thus was not used. The extent of removal of the casein was estimated on a scale of 0 to +4 (complete removal). Thus BALB/c-3T3 and the ts cells at 39 °C showed little or no cell factor activity whereas the ts cells at 32 °C, Swiss Py3T3 and BALB/c-SV3T3 showed considerable activity. Results in Table 2 also confirm that the ts Cl.1 and Cl.10 cells formed 30–50 times more colonies in agarose at 32 °C than at 39 °C, although the differences for ts Cl.X were smaller. Similarly the ts cells show decreased plating efficiencies in agar and in liquid medium at 39 °C than at 32 °C. (W.E. unpublished). In controls (not shown) ts Cl.1 cells grown at 39 °C were grown for several generations at 32 °C and these cells behaved identically to the ts Cl.1 cells grown

activity are coordinately changed with the temperature at which the cells are grown. These mutant cells, however, behave as normal fibroblasts at either temperature with respect to the regulation of their intracellular macromolecular synthesising machinery. Unfortunately it is not possible to test the ability of ts mutant cells to induce tumours *in vivo* at 32 °C and 39 °C.

The coordinate changes observed on transformation may not be reflected in every type of cell. Several non-transformed cells containing LETS protein also show significant levels of fibrinolytic activity whereas some transformed cell lines contain LETS protein but have reduced levels of fibrinolytic activity (E.P., R. O. Hynes, V. Hemmings and L. M. Franks, unpublished). Results with our mutant cells, however, suggest that at least two independent

cell functions control the transition from normal to fully transformed fibroblasts and that in these mutant cells the ability to regulate changes in some cell surface properties is uncoupled from major changes in intracellular events.

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A “permissive” effect of dexamethasone on the glucagon induction of amino acid transport in cultured hepatocytes

THE adrenal glucocorticoids have been shown to be involved in the regulation of cyclic AMP activated metabolic processes such as lipolysis¹, glycogenolysis² and gluconeogenesis³. The role of the glucocorticoids in the regulation of these processes has been termed “permissive”⁴ since it enables the maintenance of the normal responsiveness of cells to hormones which act through cyclic AMP. Glucagon is known to activate the gluconeogenic pathway of mammalian liver at several sites⁵ including amino acid transport⁶. We have studied the hormonal regulation of amino acid transport in rat liver parenchymal cells maintained in culture and report here that the synthetic glucocorticoid, dexamethasone, has a “permissive” effect on the glucagon induction of amino acid transport in hepatocytes.

Parenchymal cells from adult rat liver were isolated by a

collagenase perfusion technique and were maintained in monolayer culture in serum-free medium on collagen coated plates as described previously^{7–9}. These cells maintain many of the biochemical characteristics of adult rat liver cells for about five days in culture^{7–9}. Amino acid transport was measured by studying the uptake of the non-metabolisable amino acid α -aminoisobutyric acid (AIB).

The data presented in Table 1 established that dexamethasone, when added alone to cultures of adult rat liver parenchymal cells, has no effect on AIB transport. Experiments conducted with higher or lower concentrations of hormone and with longer or shorter times of treatment failed to demonstrate any effect of dexamethasone on amino acid transport. The addition of glucagon to the cultures resulted in a twofold increase in AIB transport. The simultaneous addition of dexamethasone and glucagon, however, produced a fourfold increase in transport. The addition of dibutyl cyclic AMP to the cells mimicked the effect of glucagon with respect to AIB transport. The simultaneous addition of dexamethasone and dibutyl cyclic AMP resulted in a further increase in AIB transport relative to those cells treated with dibutyl cyclic AMP alone.

Table 1 The effect of glucagon, dibutyl cyclic AMP, and dexamethasone alone and in combination on α -aminoisobutyrate transport

Hormone	AIB transport*
None	0.71 $\pm$ 0.02
Dexamethasone	0.70 $\pm$ 0.03
Glucagon	1.33 $\pm$ 0.06
Dexamethasone and glucagon	2.77 $\pm$ 0.11
Dibutyl cyclic AMP	1.65 $\pm$ 0.06
Dexamethasone and dibutyl cyclic AMP	3.43 $\pm$ 0.10

* nmol per mg cell protein per 4 min.

Adult rat liver parenchymal cells, which had been maintained in culture for 8 h, were treated with the indicated hormones for 12 h and then the uptake of 1 mM 2-¹⁴C-AIB in 4 min was determined. Uptake of AIB was linear for at least 8 min. The measurement of AIB uptake was conducted in a manner similar to that for the measurement of sugar uptake in cultured cells¹⁰. Briefly, the procedure consisted of rinsing the cells, which were attached to 60-mm diameter dishes, with 10–15 ml of Hanks'-Hepes medium (at 37 °C and at 8 mM with respect to glucose) and 1.5 ml of this medium containing 1 mM 2-¹⁴C-AIB was added for 4 min at 37 °C. Incubation was terminated by aspiration of the medium and the cells were rinsed with 15–20 ml of cold phosphate buffered saline. The cells were digested in 0.2 N NaOH and samples were taken for scintillation counting and protein assay¹¹. The concentrations of the hormones were as follows; dexamethasone, 10⁻⁶ M; glucagon, 10⁻⁷ M; dibutyl cyclic AMP, 10⁻⁴ M. This concentration of glucagon gave the maximum induction of transport. Each value represents the mean $\pm$ s.e. of 6 plates from 3 separate experiments.

Amino acids are major substrates for gluconeogenesis in mammalian liver⁵. The alanine cycle functions to provide carbon substrate to the liver for gluconeogenesis^{12,13}. The glucocorticoids have already been implicated in maintaining the supply of amino acids to the liver since these hormones tend to increase the release of amino acids from extrahepatic tissues^{14–16}. The data presented in this report now establish that the glucocorticoids also play an important role in the uptake of amino acids by liver since they are required for the full induction of amino acid transport by glucagon. We believe that this is the first observation of a “permissive” effect of the glucocorticoids on the hormonal induction of a transport system.

The site of action of the “permissive” effect of the glucocorticoids is not known although RNA and protein synthesis may be required¹⁷. Extton *et al.*³, have established that cyclic AMP accumulation in perfused rat liver from adrenalectomised animals after glucagon addition was normal but either hydrocortisone or dexamethasone has to be administered to observe the normal glucagon activation of gluconeogenesis. Other work² has also suggested that the site of the “permissive” effect is at a point beyond

cyclic AMP formation. Our data are in accord with these results since dexamethasone also had a permissive effect on the dibutyryl cyclic AMP induction of amino acid transport.

Our observation of the lack of any effect of dexamethasone alone on amino acid transport in hepatocytes in culture was rather unexpected since previous reports based on the administration of the glucocorticoids *in vivo* had suggested that these hormones alone can stimulate amino acid transport in hepatocytes^{18,19}. The study of hormonal regulation of metabolic processes *in vivo* is, however, always complicated by the effects of the many endogenous hormones. Thus, the use of rat liver parenchymal cells in culture for the study of hormonal regulation of metabolic processes circumvents this problem since these cells can be maintained in a completely defined serum-free medium and still maintain many of the biochemical characteristics of adult rat liver.

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Interference with normal phagosome-lysosome fusion in macrophages, using ingested yeast cells and suramin

FOREIGN bodies, including microorganisms, ingested by cultured mouse peritoneal macrophages become enclosed within phagosomes. Usually lysosome granules promptly assemble close to the phagosomes and fuse with their membranes, exposing the ingested objects to digestive enzymes^{1–3}. Much of the kinetics of phagosome-lysosome fusion and degranulation in leukocytes was described by Robineaux, Hirsch, Cohn and their co-workers^{4–7}, but the mechanisms and factors controlling them are only now beginning to emerge^{8,9}.

Some natural exceptions to the usual fusion pattern in macrophages have been observed, notably the non-fusion response to virulent *Mycobacterium tuberculosis*¹⁰ and living *Toxoplasma gondii*¹¹. These non-fusion patterns can be reversed by attaching antibody to the organisms before their ingestion^{12–14}; extensive phagolysosome formation then occurs. Such deviations and manipulations of phagosome-

lysosome fusion behaviour may help to elucidate its importance in the response of macrophages to intracellular microorganisms. The examples cited have been concerned with the parasite within its phagosome, but we now report successful inhibition of the normal fusion mechanism by a "lysosomotropic" drug (suramin), using a simple rapid method for observing fusion patterns in living cultured macrophages with the light microscope. Lysosomes were prelabelled with acridine orange^{15,16} and the phagocytes permitted to ingest live baker's yeast cells; intracellular changes were monitored in the living monolayers by dark-field fluorescence microscopy.

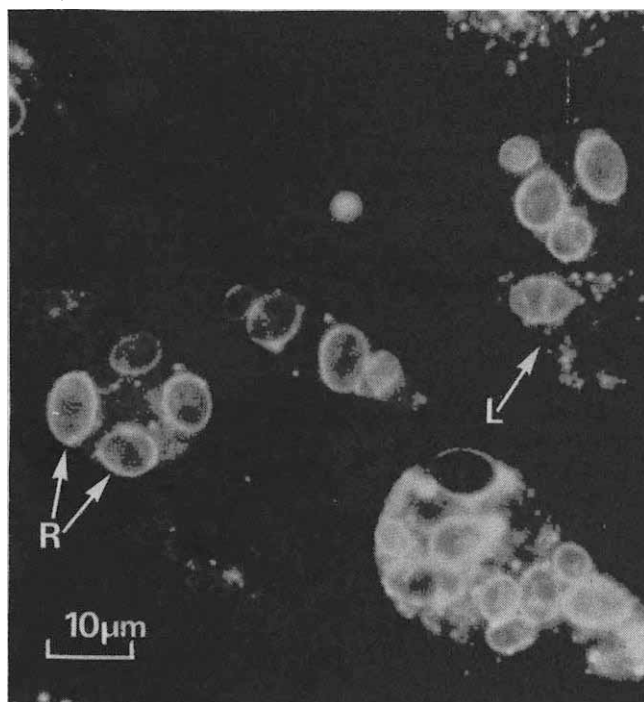


Fig. 1 Living macrophages with acridine-stained lysosomes (L) after incubation with live yeasts for 45 min. Surrounding many yeast cells are brightly fluorescent intraphagosomal "rims" (R), indicative of phagosome-lysosome fusion. Normal pattern of macrophage response.

Established cover slip monolayers of unelicited mouse peritoneal macrophages in Chang medium in Leighton tubes^{10,14,17} were given a change of medium, and 2–6 d later were washed with balanced salt solution (BSS) and exposed at 37 °C in BSS to purified acridine orange, 5 µg ml⁻¹, for 10 min. The cells were washed and infected in BSS plus 2.5% human cord serum with a suspension of fresh commercial compressed baker's yeast (*Saccharomyces cerevisiae*) (about 5 × 10⁶ yeast cells per ml; ratio to macrophages about 20 : 1). After 15 min to 6 h at 37 °C (the exposure to the yeast cells usually maintained throughout), cover slips were removed and mounted in BSS, and were examined with blue-violet light^{15,16}.

Lysosomes fused with yeast-containing phagosomes in characteristic stages during 1–2 h after the start of ingestion. The orange-fluorescing granules assembled around the unstained yeasts. Next, these periphagosomal "satellite" lysosomes disappeared and vivid orange confluent "rims" of fluorescence appeared around the individual yeasts, indicating a discharge of lysosomal contents into the phagosomes (see Fig. 1). Gradually the intraphagosomal dye permeated into the yeast cells, colouring the whole organisms green and then red; some hours later dark holes were sometimes seen, suggesting dissolution of yeast cells by digestive enzymes. These stages overlapped. Both satellite lysosomes and rims were plentiful at 15 min, but rims predominated at 45–50 min; coloured yeast cells increased and became

frequent after about 2 h, with gradual disappearance of rims. Heat-killed yeast cells were unsuitable for observing the fusion process as they took up the dye too rapidly for rims and satellites to be detected.

The monolayers were exposed to various substances that may inhibit the fusion process, before (or after) labelling of the lysosomes; yeast cells were then introduced. We examined some reputed stabilisers of lysosomes (dexamethasone sodium phosphate; chloroquine diphosphate; chlorpromazine hydrochloride); another anti-inflammatory agent (aspirin); an inhibitor of degranulation (colchicine) and also morphine hydrochloride, cyclophosphamide and cycloheximide. None inhibited noticeably the phagosome-lysosome fusion process in our test system.

Suramin (which enhances tuberculous infection in mouse macrophages)^{17,18} was then tested. It was incorporated in fresh culture medium for 6 d; the cell layers were washed, stained with acridine orange, and infected with yeast cells as before. Uptake was normal, but 45–50 min after the start of ingestion lysosome satellites were predominant, rims and

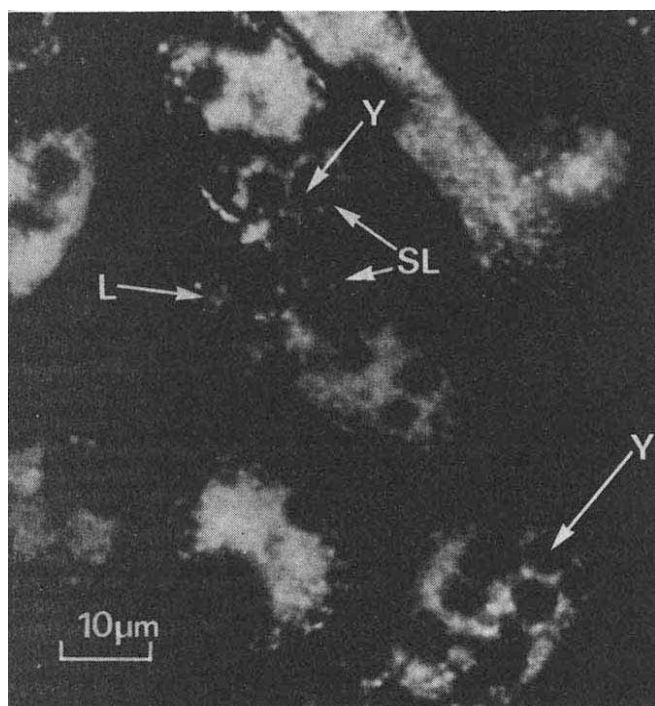


Fig. 2 Conditions as Fig. 1 but macrophages pretreated with suramin ($200 \mu\text{g ml}^{-1}$ for 6 d). Lysosomes (L) have assembled as 'satellites' (SL) around many of the ingested yeast cells (Y); fluorescent rims are absent. Non-fusion pattern of response.

coloration of the yeasts being inconspicuous (Fig. 2); concurrent controls at the same time showed mostly rims and some green or red yeast cells (Fig. 1). These appearances were interpreted as almost complete short term suppression of the normal phagosome-lysosome fusion response. They were evident after pretreatment at $200 \mu\text{g ml}^{-1}$ (maximum tolerated) to $50 \mu\text{g ml}^{-1}$, with some inhibition down to $5 \mu\text{g ml}^{-1}$. The effect was maintained for at least 5 h after 200 and $100 \mu\text{g ml}^{-1}$; however, after $50 \mu\text{g ml}^{-1}$ a few rims and coloured yeast cells appeared towards the end of this period. The drug at $200 \mu\text{g ml}^{-1}$ only partly suppressed the fusion response to heat-killed yeast cells. The suppression of fusion by suramin was confirmed by electron microscopy, prelabelling secondary lysosomes with ferritin.

The macrophage monolayers were pulsed with ferritin (10 mg ml^{-1}) for 3 h in Leighton tubes¹⁴. Next day, suramin ($200 \mu\text{g ml}^{-1}$) was added to fresh medium in half the tubes and all were incubated for 6 d at 37°C . After washing, live yeasts were added and the tubes reincubated, and the macrophages were fixed 40–50 min later^{10,19}.



Fig. 3 Ultrastructure of a macrophage with ferritin-labelled secondary lysosomes (L) after incubation with live yeast cells for 45 min. Ferritin label (F) surrounds a yeast cell wall (YW) inside the 'tight' phagosome membrane (P), indicating phagosome-lysosome fusion. A lysosome is seen fusing (LF).

In thin sections of controls fixed 45 min after the start of ingestion (Fig. 3), ferritin label was seen within about 80% of yeast-containing phagosomes. The phagolysosomal membrane was invariably applied closely to the surface of the enclosed yeast cell, that is, of a "tight" variety^{20,21}. About

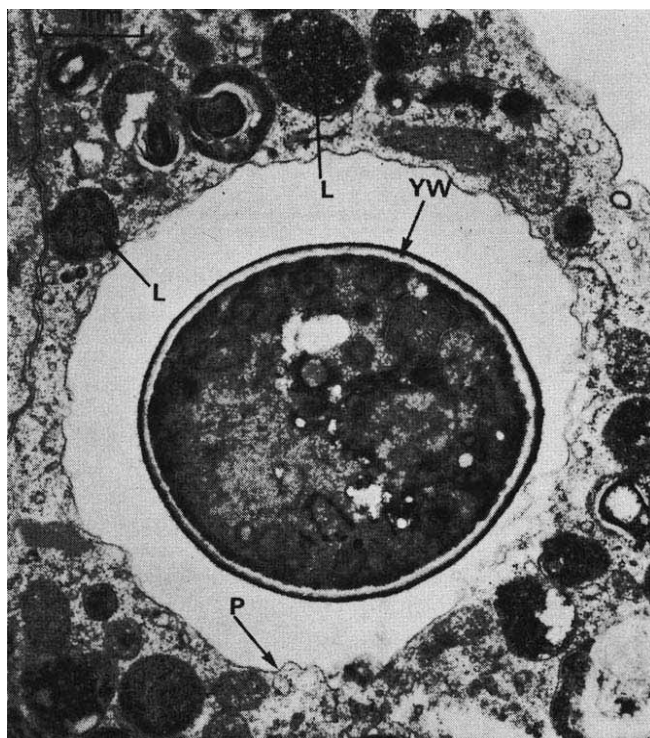


Fig. 4 Conditions as Fig. 3 but macrophages pretreated with suramin ($200 \mu\text{g ml}^{-1}$). Lysosomes (L) are labelled but ferritin is absent from zone between yeast wall (YW) and 'loose' phagosome membrane (P). Non-fusion.

half the yeasts were "granular" in appearance, suggesting incipient degradation. In suramin-pretreated macrophages (Fig. 4) the ferritin label had entered only about 10% of phagosomes; where fusion had not occurred, the phagosome membranes were almost all of a "loose" variety^{20,21}. The yeast cells mostly appeared structurally intact. Autophagic vacuoles were present in a proportion of the macrophages pretreated with suramin at 200 $\mu\text{g ml}^{-1}$.

We infer that suramin does not interfere with the periphagosomal assembly of lysosomes, but affects the membrane fusion. Suppression of this process in leukocytes by this trypanocide has not previously been reported, and it is not clear whether any of its known properties (see refs 22–25) are relevant. Suramin is a polybasic anion and binds strongly to plasma proteins. It enters cells by endocytosis and becomes concentrated in their lysosomes. It inhibits many enzymes, including some of the lysosomal proteases, and it also inhibits ($\text{Na}^+ - \text{K}^+$)-activated ATPase. It is anti-complementary.

The live-yeast/acridine orange system may prove useful for studying the mechanisms of phagosome-lysosome fusion in cultured macrophages, and throw light on how non-pathogenic microorganisms are inactivated and disposed of—especially whether the lysosomal enzymes actually kill, or merely digest organisms inactivated by other processes. Manipulations by means of a fusion inhibitor such as suramin should add to the usefulness of such a system.

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Protective tumour cell ghosts with intact membrane markers

TRANSPLANTATION antigen-like tumour-associated surface membrane antigens (TATA) have now been prepared in solubilised or semi-solubilised form^{1–3}. The methods of preparation are, however, complicated and yields are usually poor. Moreover, protection experiments with solubilised tumour antigens have rarely been successful and have given very limited protection^{1,2,4,5}. On the other hand, all manner of devitalised cells protect in immunisation experiments against living tumour cells⁶; and it is therefore possible that interference with cell membrane integrity during solubilisation destroys tertiary and quaternary structures important for the immunogenicity of membrane antigens^{6,7}. We describe here a

simple and gentle procedure for preparing in near-quantitative yield (nucleated) tumour cell 'ghosts' with retention of protective immunogenicity. Freeze drying of 'ghost' suspensions provided a stable material which retained its protective activity for 2.5 yr when stored dry at 4 °C.

Ehrlich ascites tumour cells (EATC) were obtained from BALB/c mice inoculated intraperitoneally 8–9 d previously with 10⁶ viable (dye exclusion) EATC and were immediately mixed with an equal volume of Hanks' balanced salt solution (HBSS) containing 10⁻³ M EDTA, thus giving an homogeneous suspension essentially free of erythrocytes (RBC). After two washes in HBSS or Tyrode (5 min centrifugation at 1,000g followed by resuspension to the original volume of fresh solution), the cells were subjected to hypotonic shock, essentially as performed by Ponder in the preparation of RBC ghosts⁸; the EATC were suspended at a concentration of 10⁸ ml⁻¹ in CO₂ saturated water at 4 °C, held at this temperature for 10 min, centrifuged and resuspended in fresh hypotonic solution for another 10 min. After repeating this sequence, the suspension was washed four to five times with buffer (0.14 M NaCl in 0.01 M Tris, pH 7.2). Table 1 shows that about 50% of the cell protein is extracted together with all the EATC glucose-6-phosphate dehydrogenase, an enzyme known to be associated

Table 1 Recoveries of ghosts from EATC in terms of cell counts and comparisons of proteins and enzyme contents of ghosts and intact cells

	Intact EATC	Ghosts	% Recovery
Total cell counts ($\times 10^6$)	1.44 $\pm$ 0.08	1.34 $\pm$ 0.09	93
Protein (mg per 10 ⁶ cells)	211 $\pm$ 21	95 $\pm$ 30	45
Total ATPase*	191 $\pm$ 41	194 $\pm$ 43	100
5'-nucleotidase*	27 $\pm$ 4	24 $\pm$ 5	100
Glucose-6-phosphate dehydrogenase†	1.82 $\pm$ 0.41	Nil	Nil

Ghosts can be counted as readily as intact cells and would be regarded as viable by dye exclusion although they do not form tumours when injected into mice.

* μmol phosphate liberated per h per 10⁶ cells.

† μmol NADPH produced per min per 10⁶ cells.

with cell cytoplasm⁹. On the other hand, ATPase⁺ and Na⁺ K⁺ ATPase, enzymes occurring in association with membranes^{10–12}, were retained quantitatively in the ghosts. Although 5'-nucleotidase has been shown to be membrane-bound in rat liver^{11,12}, in EATC its activity proved to be too low to be of value as a membrane marker.

Phase contrast microscopy of the EATC 'ghosts' showed a slightly enlarged and still nucleated cell with an otherwise 'empty bag' appearance. Acridine orange (AO) staining^{13,14} (using two drops of cell suspension with two drops of a 0.02%

Table 2 Quantification of membrane immunofluorescence produced by reacting fluorescein-labelled goat anti-mouse Ig with known numbers of EATC (or ghosts) incubated previously with rabbit anti-EATC serum

Rabbit anti-EATC (dilution)	Fluorimetric readings		
	Original EATC	Ghosts	Control
1:5	0.95 $\pm$ 0.05	0.96 $\pm$ 0.05	0.03
1:10	0.82 $\pm$ 0.04	0.80 $\pm$ 0.05	0.03

5 $\times 10^6$ cells were used per test and the carefully washed fluorescent cells were finally suspended in 2 ml Tris-Triton X-100. Control cells were treated with equivalent dilutions of normal rabbit serum in place of the rabbit anti-EATC serum. Fluorimetric readings were taken within 1 h of completing the tests.

aqueous solution of AO) showed under ultraviolet light that the ghosts had retained the green-fluorescing nucleus of normal EATC. The plasma membrane and a fibre-like fine network in the cytoplasm could be seen in dull red-brown outline. The usually bright orange-fluorescing cytoplasmic granules of intact EATC, however—most likely lysosomes—had disappeared, confirming the leakage of cytoplasmic contents already indicated by the chemical and enzymic tests. Yet the original cell shape was well maintained in ghosts stored in the isotonic Tris-NaCl buffer. This, in conjunction with retention of membrane enzymes, suggests remarkable retention of structural integrity and retention of an at least partially intact membrane system. Freeze-dried ghosts had a somewhat crumpled appearance, but still showed the essential structural features.

The epitopic antigen densities of the ghosts were compared with those of the intact cells by a quantitative membrane immunofluorescent method (R.A. and L. Waft, unpublished). Briefly, aliquots of 5×10^6 washed EATC (or ghost equivalents) were first reacted with rabbit anti-EATC serum and, after three washes in Hanks' balanced salt solution (centrifugation at 1,000g for 5 min), with fluorescein-conjugated anti-rabbit IgG. After further washings the cells were finally suspended in Tris-Triton X buffer (a mixture of equal volumes of 1% Triton X-100 in water and 0.04 M Tris-HCl, pH 8.0). This yields a stable fluorescent cell suspension which can be read in a conventional fluorimeter (Aminco-Bowman). Table 2 shows that equivalent numbers of intact EATC and EATC 'ghosts' gave identical readings, whereas controls (immune serum replaced by an equivalent dilution of normal rabbit serum) gave readings of about 3% of the test readings. These tests therefore indicate retention of the full surface complement of antigens demonstrable by the rabbit anti-EATC serum used.

Retention of protective anti-EATC surface immunogenicity was further established by immunising three groups of mice respectively with X-irradiated but otherwise untreated EATC (E*)^{6,15}, with ghosts and with the extract concentrates from preparing the ghosts (Ex). Each mouse received two spaced (2 weeks) injections of 10^8 E* or ghosts, and the third group the extracts of twice that number of EATC. One week after the second injection all the mice were challenged with freshly collected tumour cells (viability >95% by dye exclusion), either EATC or cells from an unrelated tumour (methylcholanthrene-induced, MC). Table 3 shows that immunisation with EATC ghosts protected as well as did the E*, whereas the extracts of even twice the number of EATC provided no protection whatever. Table 3 shows that the unrelated tumour cells grew as well in the EATC-injected as in the uninjected control mice. The specificity of the reaction was confirmed by lymphoid cell transfer experiments¹⁶. Draining node cells from mice injected with E*, ghosts or Ex were collected 5 d after the last subcutaneous injection, mixed with 5×10^6 EATC or 10^6 MC tumour cells (lymphoid cell-tumour cell ratio 100:1) and injected subcutaneously into groups of normal mice. The control groups received the two types of tumour cells

Table 4 Lymphoid cell transfer tests

Lymphoid cells from mice immunised with:	Admixed tumour cells	Tumour takes in:	
		Test	Control
E*	5×10^6 EATC	1/12	6/6
Ghosts	5×10^6 EATC	0/6	6/6
Ghosts	10^6 MC	6/6	6/6
Ex	5×10^6 EATC	6/6	3/3

Lymphoid to tumour cell ratio in the injected mixtures, 100:1.

alone. Table 4 shows that EATC tumour formation, but not MC tumour formation, was prevented by lymphoid cells from mice immunised with E* or EATC ghosts. As expected, lymphoid cells from mice immunised with Ex failed to prevent EATC tumour formation.

The fact that an essentially cytoplasm-free nucleated cell membrane bag has been found quantitatively to retain surface antigens of the intact cell and has proved fully immunogenic in protection experiments, whereas the cytoplasmic cell sap has proved completely inactive, suggests that (at least in EATC) TSTA (or TATA) are closely and probably exclusively associated with the plasma (and possibly other) cell membranes, whereas the cytoplasm is free of TSTA (TATA). Ghosts of the type prepared here may prove useful not only for protective immunisation, but also as a more satisfactory starting material than intact cells in cell-antigen purification. A fully immunogenic intermediate of this type may also help clarify features involved in preserving protective tumour immunogenicity during purification.

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Table 3 Protective immunisation in BALB/c mice

Immunising dose (Cell no. per subcutaneous injection)	Challenge (No. of viable tumour cells interperitoneally)	Tumour takes in:	
		Test	Control
10^8 E*	10^6 EATC	0/12	6/6
10^8 E*	10^6 MC	6/6	6/6
10^8 ghosts	10^6 EATC	1/18	6/6
10^8 ghosts	10^6 MC	6/6	6/6
Ex from 2×10^8 EATC	10^6 EATC	11/12	6/6
Ex from 2×10^8 EATC	10^6 MC	5/6	5/6

E*, X-irradiated EATC (10,000 rad).
Ex, combined concentrated EATC extracts obtained during the preparation of EATC ghosts.
MC, methylcholanthrene-induced tumour cells.

Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex

THE major histocompatibility (H) antigens of higher animals show extreme genetic polymorphism equalled, in higher vertebrates, only by that associated with the immunoglobulins¹. Maintenance of such a high rate of variability implies

evolutionary advantage for heterozygotes in the HL-A system for man, or at the H-2 gene complex in mice^{2,3}. We propose a possible selective mechanism, based on the realisation that immunological surveillance function (defined here as recognition and elimination of modified host cells by sensitised thymus-derived lymphocytes (T-cells) may be considerably enhanced in mice heterozygous at the H-2 gene complex.

This idea arose from experiments with two of the most prevalent naturally occurring virus infections of mice, lymphocytic choriomeningitis (LCM) and ectromelia (mouse pox). In both diseases immune T cells seem to be sensitised to altered self antigens⁴⁻⁷, the self components involved being specified at, or near to, either of the two loci (H-2K or H-2D) coding for the major transplantation antigens. Whether viral and H-2 components are in some way complexed, or more long range modification of H-2 antigens is induced by the infectious process, is neither known nor central to the present argument.

Table 1 Cytolytic activity of LCM-immune spleen cells for H-2^k virus-infected L cells, or L-929 fibroblasts

Mouse strain	H-2K	H-2D	Lytic units*	% Activity
CBA/H	kk	kk	6.5×10^4	100
CBA/H $\times$ C57BL F ₁	kb	kb	9.0×10^4	72
B10.A	kk	dd	16.0×10^4	40
C57BL	bb	bb	$> 1,000 \times 10^4$	<1

* Number of LCM-immune spleen cells necessary to cause 33% specific ⁵¹Cr release from 5×10^4 (cells per assay well) LCM virus-infected targets^{4,8}. Mice were injected intravenously with 2,000 LD₅₀ WE3 LCM virus 8 d before.

The evidence for this 'altered self' hypothesis is as follows⁴⁻⁷: First, lysis of LCM virus-infected targets by sensitised T cells occurs only if the two cell types are compatible for either one parental H-2 haplotype, or at H-2K or H-2D. Identity of immune response genes (I region) is neither sufficient nor necessary. Second, results of selective proliferation studies *in vivo* and 'cold-target' competitive inhibition experiments *in vitro* indicate that F₁ mice generate LCM-immune T cells of at least two specificities, each directed against altered self characteristics of one parental H-2 haplotype. Third, similar experiments using H-2 recombinant mice also show that homozygotes possess a minimum of two T cell specificities, associated with H-2K or H-2D. Heterozygotes would thus generate T cells of at least four specificities, sensitised to altered self antigens coded for at the H-2K and H-2D loci of each parental haplotype. The total range of the T cell response in the F₁ may thus be

Table 3 Susceptibility of H-2 different mice sharing the C57BL genetic background

	log ₁₀ intracerebral virus dose	B10.A (H-2 ^a)	B10.A $\times$ C57BL F ₁	C57BL (H-2 ^b)
% Mortality*	3 2 1	10 10 10	40 60 90	10 30 60
Days to death	3 2 1	9.5 10.0 9.0	8.5 ± 0.6 $7.5 \pm 0.3^\dagger$ $7.9 \pm 0.3^\ddagger$	11.5 8.5 ± 0.6 9.3 ± 0.1

* Groups of 10 adult ♂ mice were used.

†, $P > 0.05$.

‡, $P < 0.001$.

as much as twice that possible in either parental homozygote.

Comparison of cytotoxic T cell activities *in vitro* provides support for this concept. Repeated experiments have shown that compatibility (between T cell and target cell) at one H-2 haplotype confers at least 70% of the cytolytic capacity found in the homozygous situation⁴ (that is, full compatibility between homozygous killer T cell and target cell). Results for some of the mouse strains used in the *in vivo* experiments described below are shown in Table 1. The possible response in the F₁ associated with both haplotypes could thus be in excess of 140% of that occurring in either parent.

Enhanced T cell responsiveness in F₁ mice may also be shown *in vivo*. The LCM model used here is paradoxical in so far as our general argument is concerned, as it reflects a fatal immunopathological process directed against virus-infected cells in the central nervous system. The same response to virus growing in lung and liver results in immune elimination and recovery⁹, the normal consequence of cell-mediated immunity¹⁰. Mice injected intracerebrally with a low dose of viscerotropic (WE3) LCM virus die with severe neurological symptoms in 7-9 d. Death seems to reflect damage to virus-infected meningeal and choroid plexus cells caused by H-2 compatible immune T cells^{5,11}, LCM virus itself being of minimal pathogenicity. Inoculation of a large dose of viscerotropic virus intracerebrally, however, often results in survival. An explanation for this seemingly high dose paralysis^{12,13} is that administration of large amounts of virus leads to more extensive growth in viscera, with resultant recruitment of sensitised T cells to peripheral sites and reduced neuropathology mediated by T cells.

Heterozygotes were consistently more susceptible than were parental homozygotes to intracerebral inoculation with WE3 LCM virus, the effect being apparent at all dose

Table 2 Susceptibility of parental and F₁ mice to intracerebral WE3 LCM virus

	log ₁₀ intracerebral virus dose	BALB/c (H-2 ^a)	BALB/c $\times$ C57BL F ₁	C57BL (H-2 ^b)	C57BL $\times$ CBA/H F ₁	CBA/H (H-2 ^k)
% Mortality	3* 4 3 2 1	25 13 88 100 63	100 100 100 100 100	40 40 13 88 100	100 100 100 100 100	40 63 88 100 100
Days to death	3* 4 3 2 1	$7.3 \pm 0.3^\dagger$ 8.5 $8.7 \pm 0.4^\S$ $9.2 \pm 0.4^\S$ $8.8 \pm 0.3^\ddagger$	$8.0 \pm 0.3^\S$ $7.6 \pm 0.4^\S$ 7.5 ± 0.2 $7.8 \pm 0.1^\P$ $8.1 \pm 0.1^\P$	$10.3 \pm 0.6^\P$ $11.0 \pm 0.9^\P$ 13.0 $11.0 \pm 0.5^\P$ $10.7 \pm 0.6^\P$	$7.1 \pm 0.1^\S$ $7.2 \pm 0.2^\P$ $7.1 \pm 0.2^\dagger$ $7.3 \pm 0.2^\dagger$ $7.9 \pm 0.2^\ddagger$	7.6 ± 0.1 10.7 ± 0.9 9.6 ± 1.0 7.8 ± 0.1 8.4 ± 0.1

* Preliminary experiment, in which 90% of CBA/H $\times$ BALB/c F₁ mice died in 8.1 ± 0.2 d. The other assays were carried out concurrently. Groups of 8-10 adult (7-9 weeks) female mice were used throughout, and mice were examined twice daily for 14 d. Virus titre was determined in outbred WEHI mice, which are susceptible to intracerebral WE3 LCM virus at all dose levels.

Differences between parent strain and F₁ mice: † $P > 0.05$; ‡ $P < 0.05$; § $P < 0.01$; ¶ $P < 0.001$.

Table 4 Severity of meningitis following intracerebral LCM virus

	log ₁₀ cells per µl CSF on day:		
	6	7	8
BALB/C	3.6 ± 0.2	4.2 ± 0.1	4.2 ± 0.1
BALB/C × C57BL F ₁	4.2 ± 0.1†	5.0 ± 0.1§	NS
C57BL	3.6 ± 0.2†	4.1 ± 0.1§	4.1 ± 0.1
C57BL × CBA/H F ₁	4.2 ± 0.1†	*4.6 ± 0.1§	NS
CBA/H	3.4 ± 0.2†	4.3 ± 0.1†	4.1 ± 0.1

Mice were injected intracerebrally with 10³ LD₅₀ of WE3 LCM virus. Samples of cerebrospinal fluid (CSF) were obtained from the cisterna magna, stained with WBC diluting fluid and counted. CSF of CBA/H mice injected intracerebrally with diluent 6 d previously contains <100 cells µl⁻¹ (ref. 5).

NS, Not sampled, as no mice surviving.

* In 8 of 12 mice sampled brain swelling, the severity of which is directly related to progression of both symptoms and the inflammatory lesion⁵, was so advanced that CSF could not be obtained.

Differences between parental and F₁ mice: †, *P* < 0.02; ‡, *P* < 0.01; §, *P* < 0.001.

levels tested (Tables 2 and 3). Furthermore, the inflammatory response, which is largely produced by T cells and is directly related to severity of symptoms^{4,5}, was both more rapid and of greater magnitude in F₁ mice (Table 4). This somewhat artefactual system serves, therefore, to illustrate that the T-cell response to virally modified cells is enhanced in heterozygotes.

Results from both *in vitro* and *in vivo* systems thus support the concept that immune responsiveness is enhanced by involvement of a greater number of T-cell specificities, directed against altered self antigens coded for at H-2K or H-2D. Such an argument provides a mechanistic basis for evolutionary advantage of both gene duplication and heterozygosity at the H-2 gene complex, in the absence of positive selection for any particular H-2 haplotype.

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Specific basophil hypersensitivity induced by skin testing and transferred using immune serum

INTRADERMAL skin testing of appropriately immunised guinea pigs results in specific hypersensitivity reactions of the 24 h delayed type. As controls, we routinely skin test non-immune animals. We have found that skin testing itself can cause specific sensitisation for delayed time course reactions in normal guinea pigs. Skin testing non-immunised animals with the protein antigen keyhole limpet haemocyanin (KLH) leads, within several days, to striking macroscopic lesions at these primary (1°) injection sites in all animals. We have termed these responses 'flare reactions', and have found on histological examination that they contain large accumulations of basophils. Overall, these flares resemble hypersensitivity reactions with a delayed time

course termed 'cutaneous basophil hypersensitivity' (CBH)¹. As proof that skin testing *per se* can specifically sensitise, we obtained serum from animals with these cutaneous basophil flare reactions and carried out intravenous transfers to non-immune recipients who then received a KLH skin test. Delayed 24 h CBH reactions which were specifically elicited by this native protein antigen, were uniformly transferred by immune serum.

Two separate preparations of KLH (Pacific Bio-Marine, Venice, California) were used for these studies. Hartley strain albino guinea pigs (250–300 g) were bred at the Division of Animal Care at Yale University School of Medicine. On day 0 animals were flank skin tested by intradermal injection of 200 µg KLH in phosphate buffered saline (0.1 ml) at one site. These primary injection sites were observed daily and macroscopic skin reactions were noted at 4 d in half of the animals, were present in all 40 of the animals at 5 d, and reached a peak at 6 d (Fig. 1). These reactions were erythematous, indurated, and seemed tender to the touch. Animals initially injected with 20 and 200 µg KLH at separate sites also had macroscopic reactions at the 20 µg site, which were maximal 6 d after intradermal injection (Fig. 1).

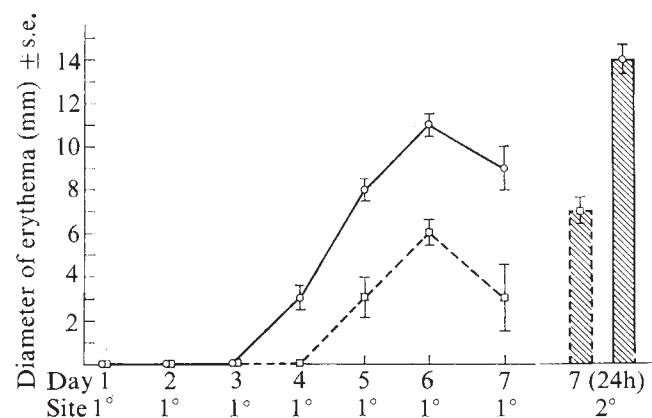


Fig. 1 Guinea pig macroscopic skin reactions at sites injected with KLH: □, 20 µg; ○, 200 µg. Primary (1°) intradermal injections were at day 0, and were read daily. Secondary (2°) injections were at day 6 and were read 24 h later.

Secondary (2°) skin tests with 20 and 200 µg KLH on the contralateral flank at day 6, resulted in large erythematous and weakly indurated reactions which began at about 4–6 h and reached a peak at 12–24 h. At this time, the 7-d-old 200 µg priming site occasionally showed superficial crusting and sloughing, and less erythema and induration than on the previous day. Histologically, this primary site (Fig. 2) and contralateral 24 h secondary skin test reactions to 200 or 20 µg KLH all had large infiltrates of basophils (Table 1) in addition to many mononuclear cells and some eosinophils. The site of skin testing performed 7 d previously with 20 µg KLH also showed CBH. It was concluded that intracutaneous injection of KLH sensitised guinea pigs for CBH, and that sufficient quantities of this antigen may have been retained at the skin test site to result in a flare of CBH at the priming site.

Table 2 shows the results of four separate experiments demonstrating serum transfer of CBH using donor animals which were immunised by intradermal injection of KLH and manifested cutaneous basophil flare reactions. Three different serum pools resulting from bleeding 7 d after priming were used for intravenous transfer of 2 ml per recipient. Two hours after transfer, recipients were skin tested with 200 µg KLH. In all four experiments, examination of these test sites showed that KLH immune sera from skin test-sensitised animals transferred macroscopic delayed (24 h) reactions containing large basophil infiltrates. Suc-

Table 1 Cutaneous basophil flares and 24 h cutaneous basophil reactions induced by intradermal injection of KLH

Time after initial injection (No. of animals)	Skin test dose	Macroscopic erythema (mm) $\pm$ s.e.	Microscopic basophils* $\pm$ s.e.
Flare reactions at priming site:			
24 h (16)	200 μ g	0	4 $\pm$ 1
48 h (6)	200 μ g	0	3 $\pm$ 1
7 d (7)	200 μ g	9 $\pm$ 1	203 $\pm$ 37
7 d (4)	20 μ g	4 $\pm$ 2	71 $\pm$ 23
24 h CBH reactions at 6 d secondary skin test site:			
7 d (13)	200 μ g	14 $\pm$ 1	103 $\pm$ 20
7 d (10)	20 μ g	7 $\pm$ 1	85 $\pm$ 18

*Skin reactions were fixed in Helly's solution, embedded in paraffin and Giemsa stained for basophil identification². Basophils were counted in five central and adjacent 180 μ m diameter oil immersion ($\times$ 1,000) fields at the uppermost dermis.

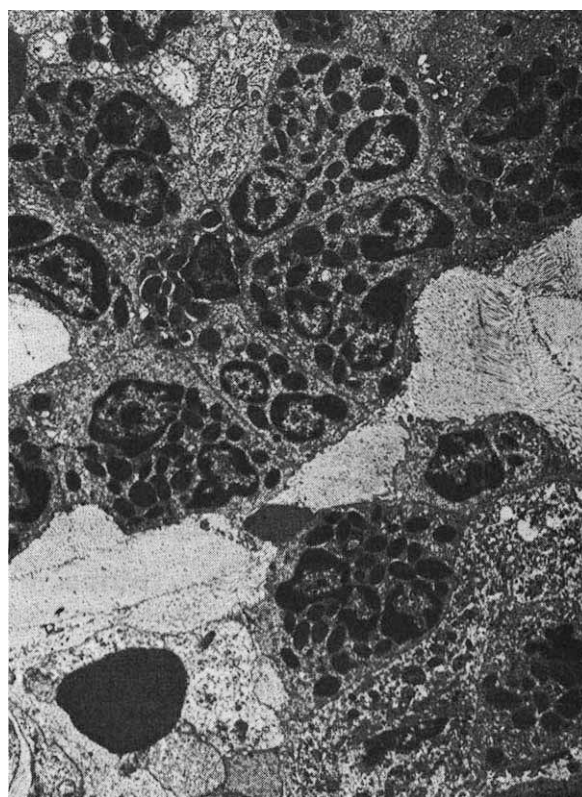
cessful CBH transfers were also accomplished with 1 week sera from KLH-primed donors which did not receive secondary skin tests. Control recipients of KLH immune sera which were intradermal skin tested with sheep red blood cells (SRBC) (5% or 20%) or irrelevant proteins (oxalalone or picryl human albumin, 200 μ g) showed no macroscopic reactions and no basophil infiltrates. KLH skin tests (200 μ g) in a variety of controls which received heterologous immune sera, media with 10% foetal calf serum (FCS), or nothing, also caused no macroscopic reactions and no basophil accumulations at 24 h (Table 2). It was concluded that skin tests can cause specific sensitisation for a reaction of the delayed type, that serum transfers of CBH to a native protein antigen can be achieved; and that serum factors contribute to cutaneous basophil flare reactions.

Our work has pointed out that serum factors are important in some delayed reactions containing significant basophil infiltrates. Thus, a component of some delayed basophil-containing reactions is hapten-specific^{2,3}, and is transferable by small quantities of immunochemically purified antibody recovered in 7S IgG₁ column fractions⁴. Antibody alone, however, is not the sole factor responsible for all delayed basophil infiltrates. We have shown that carrier-specific basophil accumulations are probably mediated by T cells⁵. Thus, B cell-derived antibodies and T cells probably function in the arrival of basophils in delayed cutaneous reactions.

Our previous studies of serum-transferred hapten-specific CBH necessarily involved sensitisation and testing with synthetic haptens conjugated to proteins and also used immunisation with adjuvants. In the present study, CBH sensitisation and accompanying cutaneous basophil flares were achieved by intradermal injection of a native protein without adjuvants. Serum obtained from these animals uniformly transferred specific delayed reactions, which were macroscopically evident in the flank skin of recipient guinea pigs, and showed numerous basophils on microscopic examination.

Jones and Mote originally described the ability of skin tests to sensitise humans for delayed reactions, and they noted flares at the primary site in nearly one third of individuals immunised by intradermal injections of hetero-

logous proteins^{6,7}. This study with guinea pigs has shown that cutaneous flares at sites of intradermal immunisation are characterised by marked basophil accumulations; we have also noted flares of basophils containing cutaneous reactions following intradermal sensitisation of humans with KLH (P.W.A. and J. E. Atwood, unpublished). In human contact reactions, which also have basophil accumulations¹, similar flares occur at primary sensitisation sites, and flares at human skin sites primed with dinitrochlorobenzene are considered the strongest criterion of normal immune reactivity⁸.

**Fig. 2** Electron photomicrograph from a primary skin test site from a non-immune guinea pig injected 7 d previously with 200 μ g KLH. A dense infiltrate of basophils is seen in the upper dermis. $\times$ 4,200.

A consideration of the ability of skin tests to sensitise is pertinent to the interpretation of specific immune reactivity following transfer of serum, cells or cell extracts (such as transfer factor, which may transfer specific immunity or act as an adjuvant⁹). It is possible that a successful transfer may in some instances depend on the ability of the transfer to augment and accelerate the sensitising or irritating potential¹⁰ of the skin test used to demonstrate the transfer. In systems in which flares can develop from skin testing, interpretation of the specificity of passively acquired

Table 2 Serum transfer of CBH to KLH following intradermal (i.d.) immunisation

Serum donor immunisation	Number of:		Material transferred	Recipient 24 h skin test reaction (200 μ g KLH)	
	Experiments	Recipients		Erythema (mm)	Basophils*
KLH (200 μ g i.d.)	4	15	2 ml sera	7 $\pm$ 0.7	82 $\pm$ 10
1% SRBC + IFA	1	5	2 ml sera	1	7
CFA	1	5	4 ml sera	0	7
None	1	3	10% FCS	0	0
None	2	6	Nothing	0	9

*See legend to Table 1. IFA and CFA, incomplete and complete Freund's adjuvant.

immunity can depend solely on an accelerated development of reactions in the first skin tests of recipients. The serum transfer of basophil sensitivity to KLH reported here was validated by the absence of reactions to various irrelevant antigen skin tests in recipients of KLH immune sera, and also by the absence of macroscopic and microscopic delayed changes to KLH skin testing in non-immune animals and recipients of heterologous immune sera. Within the context of these controls, we conclude that serum transfer of CBH to KLH can be achieved.

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Dependence of the contractile activation of skinned cardiac cells on the sarcomere length

FRANK¹ and Starling² have demonstrated that the systolic pressure developed by the heart decreased as its diastolic volume was altered in either direction from an optimum value. This law has been explained by variations of the number of cross bridges between the thin (actin) and the thick (myosin) filaments which generate force in individual cells^{3,4} in accordance with the sliding filament theory⁵.

Recent studies on skeletal muscle suggest that the release of Ca^{2+} from the sarcoplasmic reticulum (SR), which links the action potential of the surface membrane (sarcolemma) to the activation of the myofilaments, may also depend on the length of the cell. The activation of the myofilaments was found inhomogeneous at short sarcomere length (SL)⁶ except when the fibre was treated with caffeine⁷. Furthermore, less Ca^{2+} was released at shorter length in fibres injected with the Ca^{2+} -bioluminescent protein aequorin^{8,9}. The mechanism of the decrease in Ca^{2+} release, however, was not defined and no experiment was carried out on cardiac cells.

We have developed a simplified preparation to define the influence of SL on both the force developed by the myofilaments and the release of Ca^{2+} by the SR in cardiac cells. First, we have used single cells, thus eliminating the connective tissue which renders the relationship between the SL of each cell and the length of the preparation unpredictable¹⁰. Secondly, we have removed the sarcolemma of these cells by microdissection, thus placing the myofilaments and the SR in direct contact with solutions.

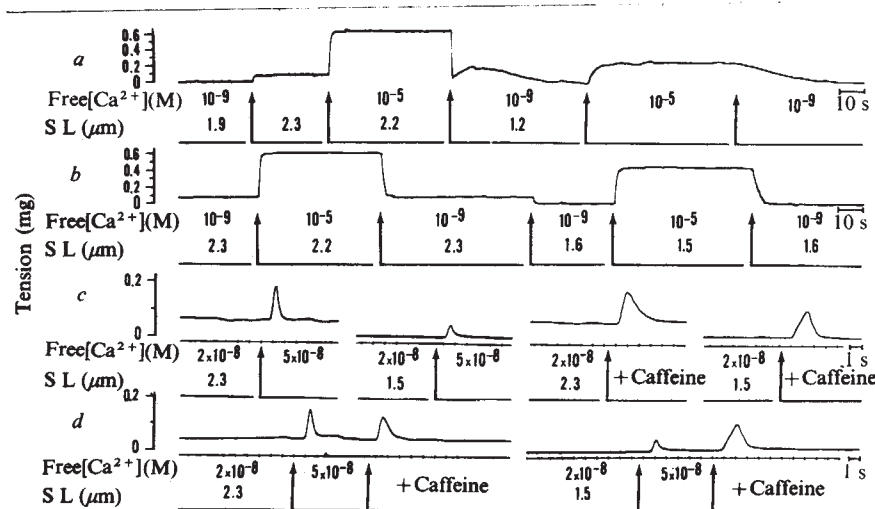
Cardiac tissue from the adult rat ventricle was homogenised into broken single cells (less than 60 μm in length and 15 μm in width) in a relaxing solution (composition given in Fig. 1). The remaining pieces of sarcolemma were removed from a broken cell by microdissection in a perfusion chamber placed on the stage of an inverted Reichert microscope¹¹. The ends of the sarcolemma-free ('skinned') cell were impaled in a direction perpendicular to the axis of the myofibrils with two glass microtools. One microtool was immobilised and the other was connected to a highly sensitive photodiode isometric transducer¹². The SL was measured on photomicrographs and on motion picture frames (100-400 f.p.s.) taken under differential interference microscopy ($\times 160$ objective with 1.30 numerical aperture) on at least 20 sarcomeres of each cell during both relaxation and contraction.

The concentration of Ca^{2+} ions (free $[\text{Ca}^{2+}]$) in the perfusing solutions was buffered with ethyleneglycol-bis (β aminoethyl ether) *N,N'*-tetraacetic acid (EGTA). We have previously demonstrated that two types of contractions can be obtained in this preparation¹¹. The tonic contractions developed by the skinned cells when the free $[\text{Ca}^{2+}]$ is increased in the presence of a large buffer capacity (that is, a high total [EGTA]) correspond to the direct activation of

Fig. 1 Arrows indicate changes of free $[\text{Ca}^{2+}]$ or of SL. When one parameter was not modified the corresponding case was left unlabelled. A single cell was used to obtain

the tracing of each row. The lengths of the cells were 45-55 μm when they were stretched to a 2.3 μm SL in relaxing solution. Their widths were: a, 12 μm ; b, 11 μm ; c, 11 μm ;

and d, 9 μm . SLs shorter than 1.5 μm were inferred from the variations of the distance between predetermined points of the cell. Striations caused by contraction bands, however, were visible at these short lengths. The total [EGTA] was 4×10^{-3} M in a and b and 5×10^{-5} M in c and d. All media contained 7 mM glucose and 18 mM Tris maleate, pH 7.0; temperature was maintained at 22 °C. The concentrations of Na_2EGTA , CaCl_2 , Na_2ATP and MgCl_2 were varied to obtain the desired free $[\text{Ca}^{2+}]$, whereas the free $[\text{Mg}^{2+}]$ was held constant at 3.16×10^{-4} M and $[\text{MgATP}]$ at 3.16×10^{-3} M. The ionic strength was kept constant at 0.16 M by appropriate addition of KCl. The following apparent association constants were used at pH 7.0: CaEGTA 4.9×10^6 M⁻¹, MgEGTA 40 M⁻¹, CaATP 5×10^3 M⁻¹, MgATP 11.4×10^3 M⁻¹. The amplitude of the tonic contractions was maximum for 10^{-6} M free Ca^{2+} and zero for a free $[\text{Ca}^{2+}]$ smaller than 2×10^{-7} M in the presence of 4.0 mM total EGTA. In contrast, phasic contractions could be triggered by a free $[\text{Ca}^{2+}]$ as low as 4×10^{-8} M in the presence of 5×10^{-5} M total EGTA. A suprathreshold free $[\text{Ca}^{2+}]$ (5×10^{-8} M) was used to trigger the phasic contractions in this study.



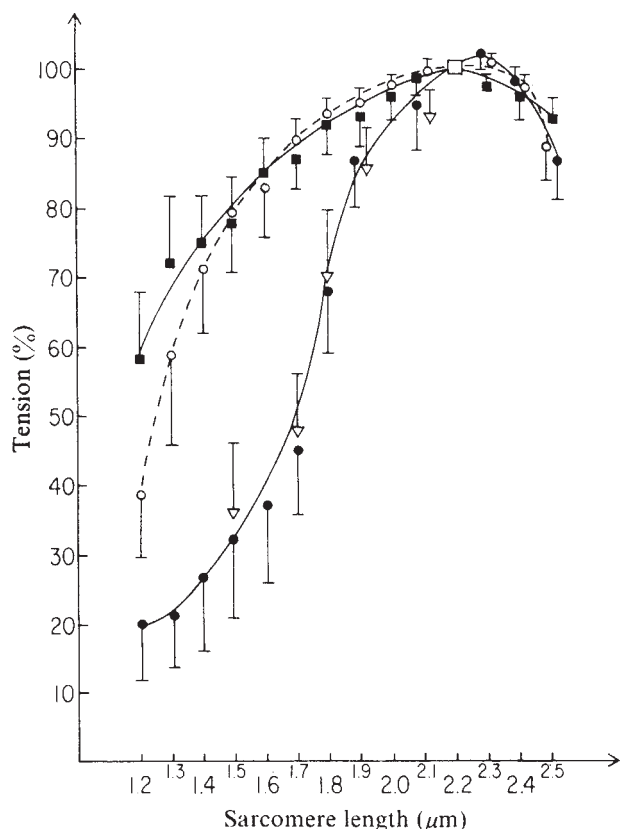


Fig. 2 Effects of the variations in SL (measured during contraction) on tonic contractions induced by direct activation of myofilaments (■) and on the phasic contractions induced by a release of Ca^{2+} from the SR triggered either by Ca^{2+} (●, △) or by caffeine (○). Tensions (plateau of tension for the tonic contractions or peak tension for the phasic contractions) were measured as percentages of tension developed by the same type of contraction at a $2.2 \mu\text{m}$ SL (□). Each point represents the mean of 12–15 measurements and each vertical bar represents 1 s.d. (shown in only one direction for clarity). Lengths of the cells were $41\text{--}63 \mu\text{m}$ when they were stretched to a $2.3 \mu\text{m}$ SL in relaxing solution. Their widths were $10 \pm 2 \mu\text{m}$ except for △, for which the widths of the cells were $5 \pm 1 \mu\text{m}$.

the myofilaments by the Ca^{2+} present in the buffer (Fig. 1a and b). In contrast, the phasic contractions triggered by a small variation of the free $[\text{Ca}^{2+}]$ in the presence of a slight EGTA buffering correspond to a Ca^{2+} -triggered release of Ca^{2+} from the SR (Fig. 1c and d). By this mechanism, a small trans-sarcolemmal influx of Ca^{2+} into the intact cell would trigger a release of Ca^{2+} from the SR sufficient to activate the myofilaments¹¹.

The effects of SL on the tonic contractions by direct activation of the myofilaments were studied in the presence of $4 \times 10^{-3} \text{ M}$ total EGTA (Fig. 1a and b). The slack SL in relaxing solution was $1.9 \mu\text{m}$ (Fig. 1a). A larger SL ($2.3 \mu\text{m}$) was obtained by stretching the cell, which resulted in the development of a resting tension. A large active tension was induced by full activation with 10^{-3} M free Ca^{2+} . To obtain SLs much shorter than the slack SL, the cell was released by rapidly moving the end not attached to the transducer, and a relaxing solution was applied (Fig. 1a). A rapid relaxation followed by a slow redevelopment of tension and a slow relaxation were observed without change of length and without variation in the spacing of the contraction bands observed at this short SL. When the relaxation was completed the contracting solution was applied, and a plateau of tension was slowly reached with no significant variation in SL ($<0.5 \mu\text{m}$). To obtain SLs moderately shorter than the slack SL, one end of the cell was simply compressed during relaxation (Fig. 1b) causing a

waving of the myofibrils, which became straight during contraction.

The compliance of the transducer ($2\text{--}3 \mu\text{m mg}^{-1}$) and the passive lengthening of damaged tissue in the areas of attachment to the microtools enabled a significant shortening of the SL to occur during contraction. Accordingly, the active tension was expressed as a function of the SL measured during the contraction rather than during the relaxation (Fig. 2). The maximum tonic tension was observed at a $2.2 \mu\text{m}$ SL and was defined as 100% tension in each cell. The tension obtained at other SLs was expressed as a percentage of this maximum value. Tensions of $58 \pm 10\%$ (s.d.) at a $1.2 \mu\text{m}$ SL and of $78 \pm 7\%$ at a $1.5 \mu\text{m}$ SL were obtained. These results are similar to those observed on skinned fibres of skeletal muscle¹³ and are very different from those observed in the intact cardiac muscle where no active tension is developed at a length 30% below optimum^{3,4}. Therefore, the Frank-Starling law^{1,2} cannot be explained entirely by a dependence of the maximum number of cross bridges between the thin and the thick filaments on the SL.

The effects of SL on the phasic contractions induced by Ca^{2+} released from the SR were studied in the presence of $5 \times 10^{-5} \text{ M}$ total EGTA (Fig. 1c and d). Short SLs were obtained by the same procedures as for the tonic contractions but with a relaxing solution containing $5 \times 10^{-5} \text{ M}$ total EGTA and $2 \times 10^{-8} \text{ M}$ free Ca^{2+} . The amplitude of these contractions decreased rapidly when the SL was decreased. It was maximum at a $2.3 \mu\text{m}$ SL ($102 \pm 3\%$ of the tension developed at a $2.2 \mu\text{m}$ SL which was chosen arbitrarily as reference) and only $32 \pm 11\%$ at a $1.5 \mu\text{m}$ SL (Figs 1c and 2). The activation of the cell was homogeneous at a $1.5 \mu\text{m}$ SL, as demonstrated by the absence of wavy myofibrils during contraction. Furthermore, no significant differences were observed in the results obtained in preparations of various widths ($5 \pm 1 \mu\text{m}$ compared with $10 \pm 2 \mu\text{m}$; Fig. 2). These observations suggest that the Ca^{2+} present in the buffer diffused homogeneously throughout the cells.

Phasic contractions were also triggered by $5 \times 10^{-3} \text{ M}$ caffeine (Fig. 1c), which releases a large fraction of the Ca^{2+} contained in the SR (ref. 14). When the SL was decreased from 2.2 to $1.4 \mu\text{m}$ the percentage decrease in amplitude of these caffeine-induced contractions was not significantly different from that of the tonic contractions elicited by the direct activation of the myofilaments (Fig. 2). This suggests that the amount of Ca^{2+} released by caffeine and the Ca^{2+} content of the SR (by inference) are not dependent on the SL in this range of SLs. A contraction was induced by $5 \times 10^{-3} \text{ M}$ caffeine at a fixed interval after the contraction induced by Ca^{2+} -triggered release of Ca^{2+} (Fig. 1d). By this method¹⁵, the amount of Ca^{2+} remaining in the SR after the Ca^{2+} -triggered release can be unmasked. The contraction induced by caffeine at a $1.5 \mu\text{m}$ SL (after the small Ca^{2+} -triggered contraction) was larger than that induced at a $2.3 \mu\text{m}$ SL (after the large Ca^{2+} -triggered contraction). Thus, a decrease in SL would not decrease the Ca^{2+} content of the SR but would decrease the amount of Ca^{2+} released by the Ca^{2+} -triggered process. Furthermore, the slow rate of tension development and the relatively rapid relaxation, which were consistently observed in the contractions induced by caffeine at short SLs, may be related to a decrease in rate of Ca^{2+} release with no modification or even an increase in rate of Ca^{2+} sequestration by the SR.

The amplitude of tonic and phasic contractions decreased when the SL was increased above 2.2 or $2.3 \mu\text{m}$ (Fig. 2), in agreement with the sliding-filament theory⁵. The apparent discrepancy between this finding and that reported in partially activated skinned fibres of skeletal muscle¹⁶ will not be discussed because SLs larger than $2.5 \mu\text{m}$ were not used in the data reported here.

In conclusion, these results suggest that decreasing the SL

below optimum results in a partial inhibition of the process of Ca^{2+} -triggered release of Ca^{2+} from the SR of cardiac cells. This mechanism may contribute to the explanation of the Frank-Starling law of the heart^{1,2}.

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Morphine antagonises prostaglandin E_1 -mediated inhibition of human platelet aggregation

COLLIER and Roy¹ have proposed that in morphine-sensitive neurones the receptor site may be adenylyl cyclase, which is physiologically stimulated by prostaglandin E_1 (PGE_1) and pharmacologically blocked by opiates. An hypothesis has been put forward that morphine exerts its analgesic effect by inhibiting the stimulation by PGE_1 of cyclic AMP formation in appropriate neurones², whereas acute side effects of opiates may be caused by stimulation of prostaglandin biosynthesis³.

We wondered if a similar receptor site for PGE_1 and morphine exists in blood platelets. The basis for this assumption is that inhibition of platelet aggregation by PGE_1 (refs 4 and 5) and PGE_2 (ref. 5) is associated with activation of adenylyl cyclase and accumulation of cyclic AMP in platelets. Therefore the final effect of PGE on platelets (that is, aggregation) is mediated through the same biochemical pathway as some central effects of PGE (for example, pain sensation). Moreover platelets have the enzymic system that synthesises prostaglandins^{6,7}.

Platelet aggregation was studied by the turbidometric technique of Born⁸. Methods for blood collection of citrate platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were as described by Han and Ardlie⁹. PPP was used to adjust the platelet count of PRP to $250,000 \mu\text{l}^{-1}$. The aggregating agents used were ADP (Sigma) at a final concentration of $2 \mu\text{M}$ and $\text{DL}(\pm)$ -adrenaline bitartrate (Sigma) at a final concentration of $50 \mu\text{M}$.

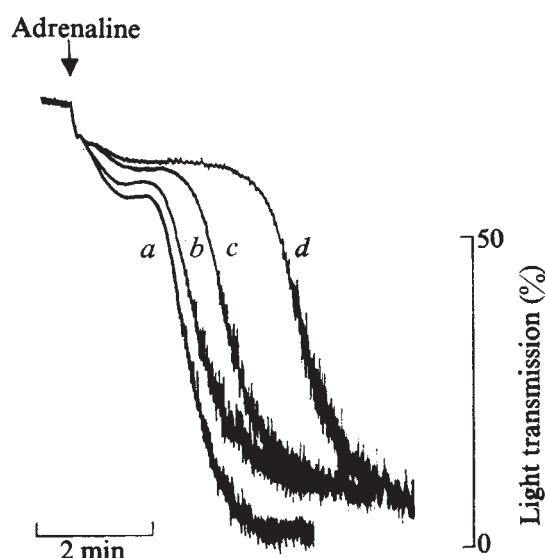
Preincubation of PRP with PGE_1 (Upjohn) at a final concentration in the range 0.028 – $0.565 \mu\text{M}$ inhibited adrenaline-induced aggregation in a dose-dependent manner. PGE_1 was added to PRP 30 s before adrenaline. Then PGE_1 flattened the slope of the first phase and delayed the appearance of the second phase of aggregation. A short

(30-s) preincubation of PRP with morphine hydrochloride (Polfa) at concentrations in the range 1.33 – $26.6 \mu\text{M}$ reduced or abolished the protective action of PGE_1 against adrenaline-induced aggregation (Fig. 1). In some platelet preparations PGE_1 caused a complete disappearance of the second phase of aggregation. In these preparations, preincubation with morphine restored the aggregating power of adrenaline. Usually the optimal active concentration of morphine was in the range 1.33 – $13.3 \mu\text{M}$. Higher concentrations than the latter often produced a paradoxical reappearance, or even enhancement of PGE_1 -dependent inhibition of adrenaline-induced platelet aggregation (Fig. 1). Some experiments with levorphanol hydrochloride (Dromoran, Roche) have shown that 5–20 times lower concentrations of the drug were needed to reach an effect equivalent to that of morphine. Incubation of PRP with morphine ($26.6 \mu\text{M}$) for up to 5 min neither changed the turbidity of PRP nor influenced markedly adrenaline-induced aggregation. In case of a distinct biphasic curve of the adrenaline-induced aggregation, morphine (2.66 – $26.6 \mu\text{M}$) either shortened or erased the intercept between both phases.

The patterns of inhibition by PGE_1 of ADP-induced aggregation were somewhat different from those observed for adrenaline. Thus, PGE_1 not only reduced the rate and intensity of aggregation but also gave rise to a delay between the addition of ADP and the onset of aggregation. All these PGE_1 -mediated protective effects against ADP-induced aggregation were reduced or abolished by a preincubation of PRP with morphine (Fig. 2). In the absence of PGE_1 , morphine ($26.6 \mu\text{M}$) did not influence ADP-induced aggregation.

The ability of PGE_1 to inhibit aggregation varied greatly in different platelet donors. Similarly, the potency of the antagonistic action of morphine against PGE_1 -mediated

Fig. 1 Prevention by morphine of the anti-aggregating effects of PGE_1 in adrenaline-induced aggregation of human PRP. For the control (a) 0.1 ml adrenaline bitartrate solution was added to 1.7 ml PRP and 0.2 ml distilled water, to yield a final concentration of $50 \mu\text{M}$ of adrenaline. To show the anti-aggregating effect of PGE_1 (d), 30 s before adrenaline was added, 0.1 ml sodium salt of PGE_1 was instilled into 1.7 ml PRP and 0.1 ml distilled water. The final concentration of PGE_1 was $0.028 \mu\text{M}$. The antagonism between morphine and PGE_1 is shown in b and c. Morphine hydrochloride in a volume of 0.1 ml was added to 1.7 ml PRP 30 s before PGE_1 , and 60 s before adrenaline. The final concentrations of morphine were $2.66 \mu\text{M}$ (b) and $26.6 \mu\text{M}$ (c). With a rise in morphine concentration the diminution of its preventive action was observed. Morphine at a concentration of $133 \mu\text{M}$ (not shown) actually enhanced the anti-aggregating effect of PGE_1 .



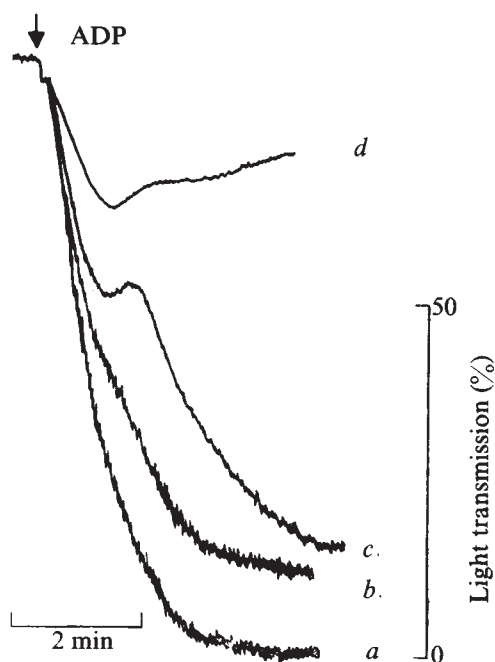


Fig. 2 Prevention by morphine of the anti-aggregating effects of PGE₁ in ADP-induced aggregation of PRP. The procedure was similar to that described in Fig. 1. Instead of adrenaline, ADP was used at a final concentration of 2 μ M. PGE₁ was used at a final concentration of 0.14 μ M. *a*, ADP; *b*, morphine (26.6 μ M) + PGE₁ + ADP; *c*, morphine (2.66 μ M) + PGE₁ + ADP; *d*, PGE₁ + ADP. A rise in morphine concentration enhanced the preventive action of morphine against the anti-aggregating effect of PGE₁.

protection of adrenaline-induced or ADP-induced aggregation differed from one plasma to another.

Summing up, the antagonism between PGE₁ and morphine similar to that described in rat brain homogenates^{1,2} may also be demonstrated in human platelets. It is probable that adenylyl cyclase is the common target for PGE₁ and morphine in platelets as it is in neurones². One of the common side effects of opiates is histaminaemia¹⁰. Basophils¹¹ and mast cells, like platelets^{4,5}, have adenylyl cyclase which is stimulated by PGE₁. Because of this, PGE₁ inhibits histamine release from basophils¹¹ and mast cells¹². Perhaps morphine blocks this PGE₁-mediated, protective mechanism and therefore histaminaemia appears.

It may be possible to use the preventive effects of opiates against the anti-aggregating action of PGE₁ for *in vitro* screening of potency of narcotic analgesics, as well as for the study on the character of opiate receptors¹³.

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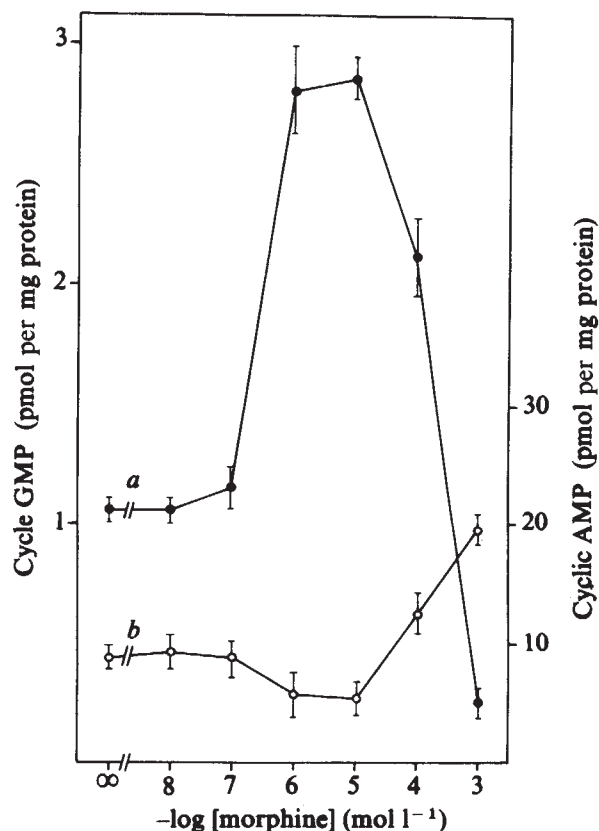
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Morphine elevates levels of cyclic GMP in a neuroblastoma X glioma hybrid cell line

THE use of cell lines derived from tumours of the nervous system as models for both neurones and glia has become well established. Clonal lines derived from mouse neuroblastoma C1300 have been shown to possess many properties characteristic of neurones¹. Several such properties are more strongly expressed in hybrids between mouse neuroblastoma and rat glioma cells²⁻⁴. They contain choline acetyltransferase²⁻⁴, clear and dense core vesicles⁵ and dopamine- β -hydroxylase⁴. In the presence of prostaglandin E₁ (PGE₁) the hybrid cells strongly increase their intracellular levels of cyclic AMP⁶. The effect of PGE₁ is antagonised by noradrenaline⁷, acetylcholine⁸ and morphine⁹⁻¹¹. The latter finding is in agreement with that in brain homogenates¹². In addition, morphine receptors could be detected in the hybrid cells¹³.

Fig. 1 Stimulation by morphine of the levels of cyclic GMP in the hybrid line 108CC15. Cells were grown in plastic dishes (Greiner, 8.5 cm diameter)^{6,15}. One hour before the experimental incubations in the presence of morphine, the growth medium was replaced by the incubation medium¹⁵. Morphine was added as described¹⁰ and the incubation ended 10 min later with the removal of the medium and addition of 3 ml ice-cold 8% (w/v) trichloroacetic acid. For control of recoveries, tritiated cyclic AMP and cyclic GMP were added. For removal of the acid, the solution was extracted 5 times with ether. The cyclic nucleotides were separated on a column of BioRad AG 1 $\times$ 2 (Cl⁻200-400 mesh, 4 $\times$ 0.5 cm). After application of the sample, the column was washed with 10 ml H₂O and eluted with 6 ml 0.05 mol l⁻¹ HCl (cyclic AMP) and subsequently another 6 ml 0.5 mol l⁻¹ HCl (cyclic GMP). After neutralisation with 3 mol l⁻¹ Tris base, the cyclic GMP fraction was rerun on regenerated columns, to remove traces of cyclic AMP. After lyophilisation, the eluates were analysed for cyclic AMP and cyclic GMP. The results are mean values $\pm$ s.d. of data obtained from 4 parallel plates. Passage number 15; 1.2×10^6 viable cells per plate; viability 92%. ●, cyclic GMP; ○, cyclic AMP.



In view of the opposing effects of cyclic AMP and cyclic GMP¹⁴, we studied the levels of cyclic GMP in the hybrid cells in response to morphine. After incubation (10 min) in the presence of the drugs under study, the cyclic nucleotides were extracted from the cells by trichloroacetic acid and separated by anion exchange chromatography. Cyclic AMP^{15,16} and cyclic GMP (P. Wunderwald, D. van Calker, R. G., J. T. and B. H., unpublished) were determined by protein binding assays.

It can be seen in Fig. 1 that morphine (0.1 to $10 \mu\text{mol l}^{-1}$) elevates cyclic GMP levels and that there seems to be a concomitant depression in basal levels of cyclic AMP. At higher morphine concentrations (0.1 to 1 mmol l^{-1}) this elevation is reversed to cyclic GMP values below those found in the absence of morphine and accompanying this there is a rise in cyclic AMP levels. The intracellular concentrations of cyclic GMP are about one order of magnitude lower than those of cyclic AMP and the absolute changes are much smaller for cyclic GMP than for cyclic AMP.

Naloxone, a potent antagonist of the action of morphine¹⁷ inhibits the elevation of cyclic GMP levels produced by morphine (Fig. 2). With naloxone concentrations two orders of magnitude lower than those of the morphine used as stimulant, levels of cyclic GMP are reduced below basal (Fig. 2). In the absence of morphine, naloxone decreases cyclic GMP and increases cyclic AMP (Fig. 2) levels, but such changes occur at concentrations (0.1 to 1.0 mmol l^{-1}) much higher than those necessary to reduce cyclic GMP levels elevated by morphine.

The elevation by morphine of cyclic GMP levels could be mimicked by levorphanol, a congener of morphine, but not by its inactive enantiomer dextrorphan (Fig. 3). Cyclic GMP levels were increased at levorphanol concentrations of 0.1 to $10 \mu\text{mol l}^{-1}$. An inhibition of this increase occurred at higher concentrations (Fig. 3). In response to levorphanol, the level of cyclic AMP changes inversely to that of cyclic GMP (Fig. 3). At high concentrations, dextrorphan reduces the basal level of cyclic GMP, while

Fig. 2 Naloxone inhibits the elevation of levels of cyclic GMP caused by morphine. 1.4×10^6 viable cells per plate (passage number 17, viability 90%) were incubated at varying concentrations of naloxone in the presence (●) and absence (■; ○) of morphine ($10 \mu\text{mol l}^{-1}$). ● and ■, cyclic GMP; ○, cyclic AMP.

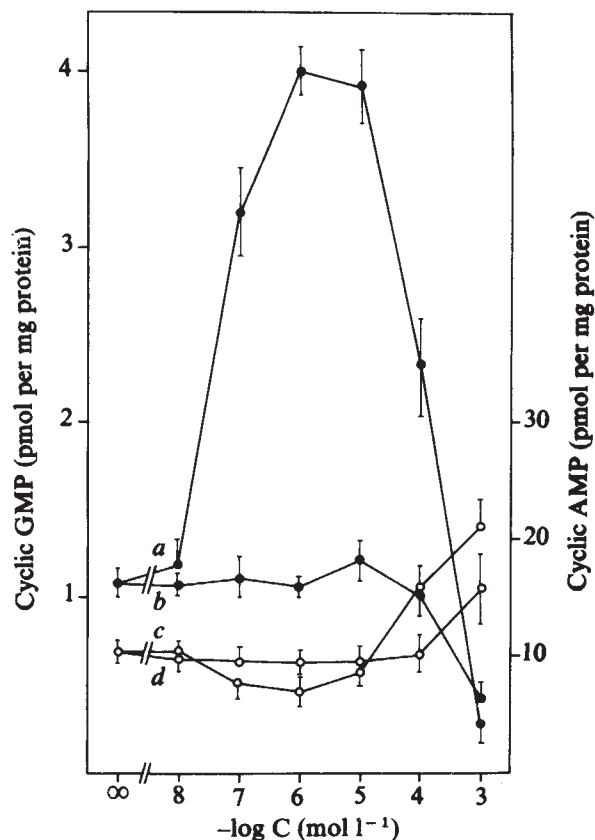
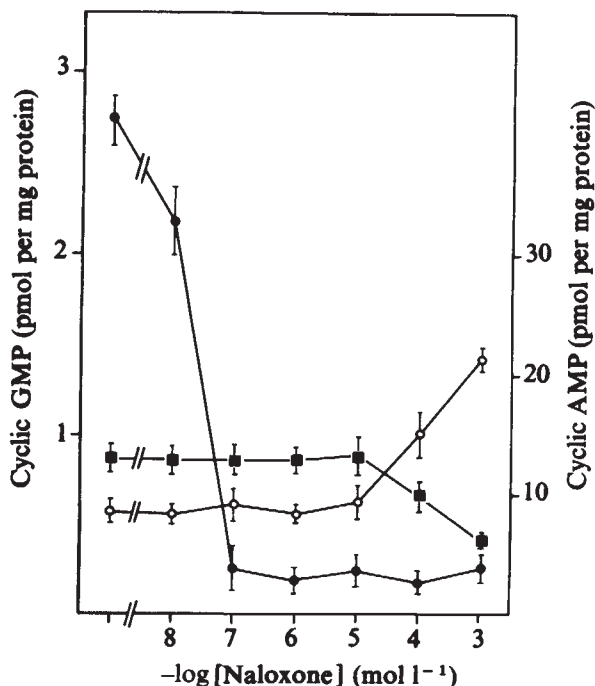


Fig. 3 Effect of levorphanol and dextrorphan on levels of cyclic GMP and cyclic AMP. Cells were incubated with levorphanol (curves a and c) or dextrorphan (curves b and d). Curves a and b, cyclic GMP; curves c and d, cyclic AMP. Passage number 12; 1.2×10^6 viable cells per plate, viability 91%. The level of cyclic GMP in the presence of $10 \mu\text{mol l}^{-1}$ morphine was 2.9 ± 0.2 pmol mg^{-1} protein.

it elevates the level of cyclic AMP (Fig. 3). In two ways the elevation by morphine of levels of cyclic GMP in the hybrid line was demonstrated to be a specific opiate action. It was antagonised by naloxone, and mimicked by levorphanol but not dextrorphan. An analogous specificity was found for the inhibition by opiates of the elevation of cyclic AMP levels by PGE_2 ^{7,10}. The doses of morphine required for a 50% increase of the cyclic GMP level (Fig. 1) and a 50% depression of the cyclic AMP level¹⁰ agree within one order of magnitude. This similarity could suggest that the depressing effect of morphine on cyclic AMP levels^{7,9,10} is perhaps mediated by cyclic GMP. In the hybrid cells noradrenaline and acetylcholine, like morphine, lower cyclic AMP levels⁷⁻¹⁰ and increase levels of cyclic GMP (R. G. and B. H., unpublished). The negative regulatory influence of cyclic GMP on cyclic AMP levels seems to be a more general phenomenon. But, the reverse hierarchy of the cyclic nucleotides is also found; at increased concentrations, morphine or naloxone cause a rise in the level of cyclic AMP whereas the cyclic GMP level is depressed (Figs 1 and 2). What is the molecular basis of these antagonisms? Most of the actions of cyclic nucleotides are mediated through the agency of protein kinase and phosphorylation reactions. Thus, it is interesting to note that such phosphorylation reactions have been shown to inhibit adenyl cyclase activities¹⁸.

Besides antagonising the effect of morphine at concentrations below $0.1 \mu\text{mol l}^{-1}$ and increasing levels of cyclic AMP at concentrations above $10 \mu\text{mol l}^{-1}$, naloxone causes a third effect. Although, alone at concentrations less than $10 \mu\text{mol l}^{-1}$ it does not depress the level of cyclic AMP below the control value, it does so in the presence of

10 $\mu\text{mol l}^{-1}$ morphine (Fig. 2): that is, it overcompensates the stimulatory effect of morphine. Experiments are under way in an attempt to elucidate the mechanism of this unusual action.

The opposite actions of morphine or levorphanol at low and high concentrations suggest that these opiate effects are mediated by receptors of high and low opiate affinity, respectively. Since, at high concentrations levorphanol and dextrorphan have the same effects, the low affinity receptor should lack stereospecificity. High and low affinity opiate binding sites of high and low stereospecificity, respectively, have been detected in brain¹⁹. Opiates do not normally occur in animals. There should therefore exist in brain at least two different natural ligands of hormone-like character, which bind to the "opiate receptors" and which are mimicked probably by different parts of the opiate molecules. We like to suggest that the actions of these endogenous ligands should be mediated by cyclic GMP and cyclic AMP, respectively. We are trying to isolate and identify these substances.

We cannot yet decide whether in opiate action the increase in levels of cyclic GMP or the depression of (hormonally increased?) cyclic AMP levels by elevated levels of cyclic GMP is the key event. This is an important issue, since it has been proposed¹² that a mechanism, whereby morphine-like drugs exert their analgesic and other effects, is the inhibition of the stimulation by PGE_1 of cyclic AMP production. But morphine also inhibits the stimulation of cyclic AMP formation caused by other agents⁷. Thus, the antagonisms between PGE_1 and morphine may or may not be a fortuitous combination of little physiological significance.

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2-Hydroxy-oestradiol-17 β as a possible link in steroid brain interaction

IN spite of wide evidence for their existence, mechanisms of steroid-brain interaction are poorly understood. It has been reported that hydroxylation of oestrogens occurs in the liver^{1,2} and that hydroxylated oestrogens such as 2-hydroxy-oestradiol-17 β (2-OHE₂) are strong competitive

inhibitors of the methylation of catecholamines by catechol-O-methyltransferase³. We report for the first time the action of 2-OHE₂ on the brain. Our experiments demonstrate that 2-OHE₂ lowered plasma levels of the anterior pituitary luteinising hormone (LH). A response was recorded from the amygdala, which accumulate gonadal steroids^{4,5} and participate in the regulation of the anterior pituitary.

Nine adult miniature pigs (Göttingen strain) were orchidectomised at least 4 weeks before the experiment, and kept in daylight and otherwise standard conditions. Stainless steel tubing (22 G) with inner exchangeable tubing (27 G) was stereotactically and bilaterally implanted into the amygdala. Histology showed that in seven animals the tip of the implant was located in the basolateral and cortico-medial amygdala and in two animals outside the amygdala. Two weeks after the implantation, a catheter was placed into the vena jugularis externa for the collection of blood samples. Treatment started 5 d later. Each individual unanaesthetised animal was microinjected by way of the inner cannula using a 10 μl Hamilton syringe (2 μl single injection; stock was made up from 7 ml 0.9% NaCl and 3 ml 20% ethanol (EtOH)). Several days later 2-OHE₂ (2 μl NaCl-EtOH containing very freshly dissolved 60 ng 2-OHE₂) was microinjected. This was followed by another NaCl-EtOH control treatment. Microinjections lasted no longer than 1–2 min. Blood samples (2 ml) were collected at 15 min intervals from each animal: nine samples 120–0 min before, eight 15–120 min and eight 135–240 min after the microinjection. At 24 and 48 h after injection, blood was collected again at 15 min intervals for 90 min. A 4-day recovery was allowed between treatments. Plasma LH was analysed by double-antibody radioimmunoassay⁶. In addition to the two control animals with implants just outside the amygdala, elaborate experiments were performed to exclude leakage to other brain regions, the pituitary or the peripheral circulation. These included application of radioactive steroids into rat and pig brains, estimations of the distance of diffusion, as well as radioimmunological determination of circulating steroid levels after steroid microinjections identical to those of 2-OHE₂ (N.P. and F.E., unpublished).

Table 1 shows that 2 μl NaCl-EtOH had no effect on plasma LH levels. 2-OHE₂ decreased plasma LH significantly at 13 out of 27 points of time. The number of animals with a significant decrease in plasma LH after injection increased with time. Microinjection of any substance outside the amygdala did not alter plasma LH significantly. An individual response during the entire experiment is shown in Fig. 1. The consistent changes in plasma LH after the microinjection of 2-OHE₂ into the amygdala clearly show that this compound can alter brain pituitary function. The well known fact that oestrogens concentrate in the amygdala^{4,5} and observation that increased catecholamine content in the amygdala is associated with lowered plasma LH levels⁷ are consistent with the mechanisms of action of

Table 1 Effect of microinjections of 2-hydroxy-oestradiol-17 β (2-OHE₂) on plasma LH levels in orchidectomised adult miniature pigs

Time after microinjection (h)	NaCl-EtOH* (60 ng-2 μ l NaCl-EtOH)				2-OHE ₂			
	<i>n</i>	NC	<i>n</i>	<i>D</i>	NC	<i>n</i>	NC	
0-2	7	7	7	2	5	5	5	
2-4	7	7	7	3	4	5	5	
24-25.5	7	7	7	4	3	4	4	
48-49.5	7	7	6	4	2	2	2	

* 2 μl NaCl-EtOH (7 ml 0.9% NaCl + 3 ml 20% ethanol).

n, Number of animals. Two animals in which the tips of cannulas were outside the amygdala are not considered in the Table. They did not cause a change.

D, Decrease in plasma LH levels. Significant (at least $P < 0.05$, Student's *t* test) when compared with the control periods.

NC, no change.

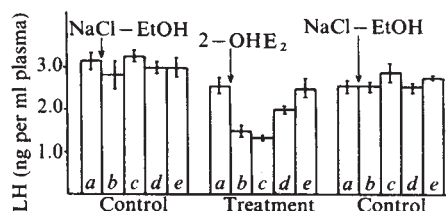


Fig. 1 Plasma LH (mean $\pm$ s.e.m.; LER-786-3) after microinjection of 2-OHE₂ into the amygdala of an individual orchidectomised adult miniature pig. NaCl-EtOH: 2 μ l of a stock made from 7 ml 0.9% NaCl and 3 ml 20% EtOH; 2-OHE₂: 2 μ l NaCl-EtOH containing 60 ng OHE₂; number of samples per block: seven to nine, obtained at 15 min intervals. Each column (b-e) was compared with the corresponding control period (a) by Student's *t*-test. 2-OHE₂ treatment resulted in significant decrease in columns (b) and (c), ($P \leq 0.001$) and in column (d), ($P \leq 0.01$). a, 0-2 h before microinjection; b, 0-2 h after microinjection; c, 2-4 h after microinjection; d, 24-25.5 h after microinjection, e, 48-49.5 h after microinjection.

2-OHE₂ (ref. 3). It remains to be established whether it is crossing the blood-brain barrier or is synthesised in the brain, or both. It is also necessary to determine whether 2-OHE₂ actions occur in other oestrogen-concentrating brain areas.

2-Hydroxy-oestradiol-17 β was kindly provided by Dr H. Breuer, Bonn, Germany, the LH antiserum by Dr G. D. Niswender, Fort Collins, USA and the LH standard by Dr L. E. Reichert, Atlanta, USA. This work was supported by the Deutsche Forschungsgemeinschaft.

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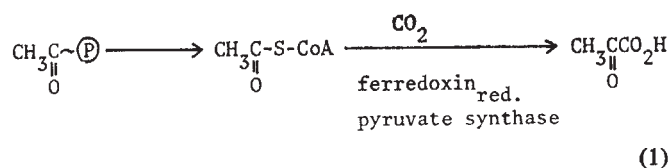
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Amino acid synthesis through biogenetic-type CO₂ fixation

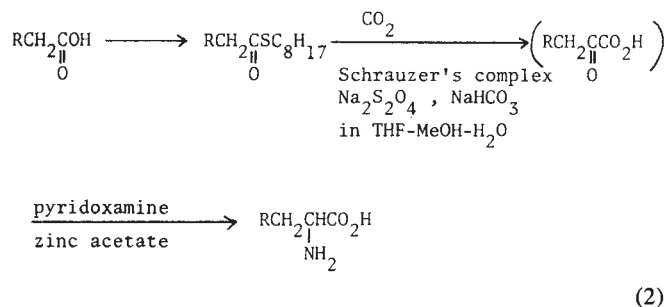
IN bacterial photosynthesis, reductive CO₂ fixation was thought to proceed through activation of carboxylic acid by coenzyme A followed by the reductive incorporation of CO₂ in the presence of reduced ferredoxin (a kind of non-haem iron-sulphur protein) and an enzyme, pyruvate synthase, as shown in equation (1). The reaction takes place *in vitro* in the same way using CoA, reduced ferredoxin and pyruvate synthase¹.



During attempts to prepare some artificial photosynthetic models, we have discovered a similar biogenetic-type reaction which uses a simple catalyst modelling ferredoxin activity. As catalysts, we used some known iron-sulphur

complexes such as Schrauzer's complex², Yang's complex³, and Holm's complex⁴.

We now wish to report that completely artificial catalytic systems, one of which consisted of a simple alkyl mercaptan, one of the ferredoxin models, and an inorganic reductant, also carried out the reaction¹. Moreover, the CO₂ fixation products, α -keto acids, were successfully converted to the corresponding α -amino acids on treatment with pyridoxamine. It is worth noting that all of these reactions can be carried out in one step as shown in equation (2).



Gaseous CO₂ was bubbled into a solution consisting of tetrahydrofuran (30 ml), methanol (15 ml), water (7.5 ml), synthetic iron-sulphur complex (500 mg, 9.2×10^{-4} mol), sodium hydrosulphite (10 g, 6×10^{-2} mol) and sodium bicarbonate (500 mg, 6×10^{-3} mol). The solution turned dark green, presumably as a result of the formation of the reduced iron-sulphur complex soluble in the solvent. Now, *n*-octyl thiophenylacetate C₆H₅CH₂COSC₈H₁₇ (264 mg, 10^{-3} mol), pyridoxamine dihydrochloride (48.3 mg, 2×10^{-4} mol), potassium hydroxide (22.4 mg, 4×10^{-4} mol) and zinc acetate (55 mg, 2.5×10^{-4} mol) were added to the solution. CO₂ bubbling was continued under efficient reflux and with stirring at room temperature. After 9 h bubbling, the reaction mixture was extracted three times with ether. The aqueous solution was subjected to anion exchange resin chromatography using quarternary ammonium resin.

The products were absorbed on the resin, and eluted with aqueous HCl. The product appeared in the first 1 N-HCl elute, and behaved as authentic phenylalanine, giving a thin-layer chromatography (TLC) spot (silica gel) of the same R_f value with phenylalanine and the same red-purple colour as phenylalanine when treated with ninhydrin. On treatment with CH₂N₂, products gave the same TLC R_f value (silica gel) and red-purple colour as the methyl ester of phenylalanine, and also the same retention time on gas chromatography. Phenylalanine formation in the present reaction was confirmed through coinjection with authentic methyl ester of phenylalanine, infrared and mass spectroscopy.

Thus, the synthesis of phenylalanine by CO₂ fixation with completely artificial (non-enzymatic) catalysts was achieved without addition of any subcellular material. The formation of phenylpyruvic acid was also fully confirmed as phenylhydrazone (TLC, infrared spectroscopy and nuclear magnetic resonance) and its methyl ester (TLC). This strongly indicates that phenylalanine was formed from phenylacetic acid (or more favourably from its thiol ester) by way of phenylpyruvic acid (as a reductive CO₂ fixation product) by amino transfer from pyridoxamine as shown in equation (2).

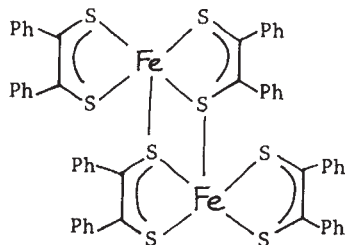
Alanine and leucine were similarly synthesised from corresponding thiol esters of carboxylic acids; the structures of amino acids thus formed were ascertained by TLC and vapour phase chromatography (VPC) after converting them into the appropriate derivatives.

Control experiments were carried out where each of the important components in turn was omitted from the complete system which consists of thiol ester of phenylacetic acid, Schrauzer's complex, sodium hydrosulphite, CO₂ gas,

and amino transfer reagents—pyridoxamine dihydrochloride, potassium hydroxide and zinc acetate. In these control experiments, no trace of phenylalanine was detected. These results show that all of the important components are necessary for the synthesis of an α -amino acid.

The possibility of contamination especially with α -amino acid and/or α -keto acid in all reagents used (whether prepared by us or not) was excluded.

The iron-sulphur complex used in the present non-enzymatic system involves the so-called "Schrauzer's complex," first synthesised by G. N. Schrauzer and its structure in the oxidised state is shown in equation (3).



Schrauzer's complex

(3)

The complex showed the same melting point and characteristic infrared spectrum as reported². The insoluble complex was easily reduced with sodium hydrosulphite and the reduced form was soluble in the present solvent. The reduced form has $\lambda_{\max}$ at 314 and 443 nm.

The electronic spectra of Yang's complex³, Holm's complex⁴ and cysteine-containing peptides⁵ were shown to be very similar to those of ferredoxin, and characteristics of reduced iron-sulphur proteins such as their ENDOR and ESR spectra⁶, magnetic susceptibility⁶ and redox potential⁴ were approximately reproduced by artificial iron-sulphur complexes. Our success in a fully artificial CO_2 fixation using Schrauzer's complex may be caused by the similarity of electronic structure between artificial iron-sulphur compound and iron-sulphur protein.

The present non-enzymatic reaction is novel and will aid understanding of the chemical nature of the biological CO_2 fixation process. It is true that this non-enzymatic reaction gives only a low yield, but it is still close to the yield of *in vitro* enzymatic CO_2 fixation. The yield of pyruvic acid was 0.66% based on acetyl phosphate used. The yield in the present reaction was 0.3% based on pyridoxamine used. Some attempts to improve the yield of non-enzymatic CO_2 fixation are now in progress.

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subject of extensive research. Seasonal rhythms in experimental laboratory animals living in a constant laboratory environment, however, have been considered improbable. The first report on seasonal rhythms in laboratory animals was made in 1958 by Gunn and Gould⁷, who reported a seasonal cycle in the uptake of ^{65}Zn by the dorsolateral prostate of the male rat, with elevations in February–March and June–July. Comments on the possibility of seasonality of androgenic hormones^{8,9} only emphasise that no study has been reported on the male laboratory rat to determine whether there are seasonal variations in plasma testosterone and luteinising hormone (LH) throughout the year. We began such a study in January 1973 and report data collected up to June 1974, which suggest a circannual rhythm in sex hormones in male laboratory rats living in what is apparently a constant environment.

At the time the study began, eight rats of Sprague–Dawley descent were placed in a windowless vivarium with lights on from 0600 to 1800 (eastern standard time). Temperature was maintained at $21 \pm 1^\circ\text{C}$; Purina rat chow and water were provided *ad libitum*. The animals lived in the experimental environment for 4 weeks before the first blood was collected on February 15, 1973, when the rats were 85 d old. Approximately 1 ml of blood was collected monthly by cardiac puncture under ether anaesthesia. Blood was collected between 1000 and 1200. Other rats of similar birthdate and treatment were added to the study during the first nine months to replace animals lost while collecting blood. On November 1, 1973, animals more than four months old were added to replenish the experimental group, which was badly depleted due to loss of ageing animals. Plasma testosterone and LH levels were also measured in a control group (age control) of six 90-d-old and six 100-d-old males during the spring of 1974, to evaluate the effect of age on seasonal changes in hormone levels. Testosterone concentration was measured by radioimmunoassay using a method we developed¹⁰, LH concentration by an ovine-ovine rat radioimmunoassay¹¹.

In the 18-month study of rats more than 80 d old, plasma testosterone concentration showed a significant elevation in February–March, 1973 (designated the spring surge) (Fig. 1). There was no spring surge, however, in 1974 in animals more than 180 d old, although plasma testosterone levels were elevated in the younger rats (age control) (Fig. 1). LH levels also rose significantly in the spring of 1973, but did not rise in older male rats in the spring of 1974 (Fig. 1), although the concentration of LH was elevated in the age control group.

The rise in plasma testosterone in 85-d-old rats during February 1973 and 130-d-old rats during March 1973 in our 18-month study contrasted with a report by Grota¹², who described a testosterone surge in 60-d-old rats after which the hormone decreased sharply as the animals aged. Since Grota did not report the birthdate of his experimental animals, we repeated his study in males born in the spring (April 22, 1974) and autumn (October 15, 1973).

In rats which matured in the summer, plasma testosterone levels increased at 61 d of age (June 22) and then decreased (Fig. 2), as Grota reported. In animals born in the autumn, however, plasma testosterone levels increased at 50 d of age, decreased at 60 d, and then increased sharply at 90–100 d (Fig. 2), during January 1974. Apparently the seasonal surge which we observed in our original experiment was based on the fact that in rats born in the autumn, the 60-d plasma testosterone surge was delayed until spring.

The thesis that the 60-d plasma testosterone surge is subject to seasonal regulation was tested further by examining plasma testosterone levels in successive groups of rats, reaching 60–65 d of age during each month of the year except April. A 60-d surge was noted in all months but June and December (Fig. 3), if we define a surge as $>3 \text{ ng ml}^{-1}$. Although the relatively low levels of plasma

Seasonal rhythm in plasma testosterone and luteinising hormone of the male laboratory rat

SEASONAL reproductive rhythms of animals living in natural conditions^{1–4} as well as domestic animals^{5,6} have been the

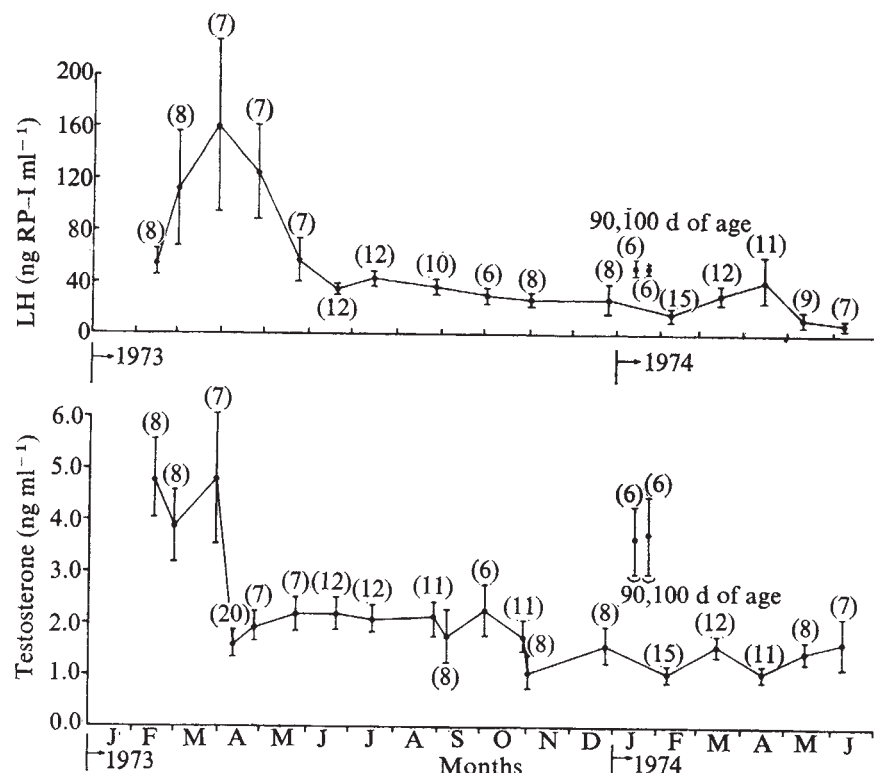


Fig. 1 Plasma testosterone (lower) and LH (upper) concentrations of mature male rats (>85 d of age) over 18 months. Each point in the figure represents ng ml⁻¹, mean $\pm$ s.e.; the number of animals tested is given in parentheses above each point. Rats were purchased from Russell Miller Farms, at 50 d of age, and reared in vivaria under constant conditions, as described in the text. Blood was collected by cardiac puncture between 1000h and 1200h under light ether anaesthesia. Testosterone and LH were measured by radioimmunoassay^{10,11}. Statistical analysis (ANOVA and Student-Neuman-Keuls test) showed that plasma testosterone was significantly increased ($P < 0.05$) during February and March 1973 over all other sampling times. Plasma LH was significantly increased ($P < 0.05$) during March 1973 over all other sampling times. In the age control group of 90-d-old and 100-d-old animals tested during January 1974, hormone levels were significantly increased ($P < 0.05$) compared with other sampling periods of older animals during 1974.

testosterone of 60-d-old rats in June did not represent a delay but rather a diminution of the 60-d surge (Fig. 2), the low levels of plasma testosterone of 60-d-old male rats in December did represent a delay in the surge, which reached a maximum in January 1974, when the rats were 90–100 d old (Fig. 2).

Rats older than 5 months did not respond in the spring, but instead their previous 60-d surge showed increasingly higher levels of plasma testosterone depending on their date of birth (compare Fig. 3, July to November). We investigated whether older rats could respond to gonadotrophins by injecting 0.1 IU human chorionic gonadotropin (HCG) per 100 g body weight into 8-month-old male rats and comparing their plasma testosterone levels over 2-h with those of HCG-injected 3-month-old rats (Table 1). Although we found testosterone concentrations consistently lower in aged rats, both groups responded significantly to HCG stimulation.

Apparently the hormonal seasonal surge was not based on testicular competence but reflected a complex regulation which is not solely dependent on environmental cues. The delay in the 60-d surge of animals born in the autumn is reminiscent of delayed sexual maturation of the field rodent during the winter¹³; in fact we recently observed a stunting of body weight during the growth of autumn-born animals

during the winter. It could be argued that the vivarium's "constant environment" contained hidden cues (in part-

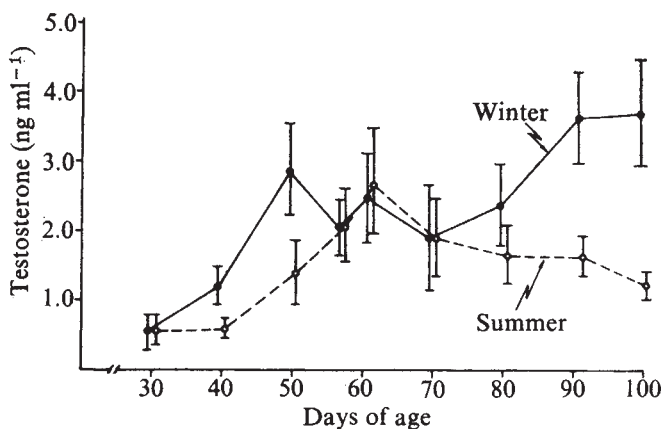


Fig. 2 Plasma testosterone concentrations of maturing male rats in summer (○) (born April 22), and in winter (●) (born October 15). Each point represents the ng ml⁻¹, mean $\pm$ s.e. of six animals. Animals were purchased from Russell Miller Farms at 21 d of age and maintained in our vivaria in constant conditions as described in the text.

Table 1 Effect of HCG on plasma testosterone levels of young and old male rats

Group	Treatment	Control sample	Time (min) after injection			
			15	30	60	120
Young	Saline	1.17 $\pm$ 0.34	1.33 $\pm$ 0.31	1.47 $\pm$ 0.42	1.66 $\pm$ 0.42	2.11 $\pm$ 0.37
Young	HCG	1.01 $\pm$ 0.07	1.85 $\pm$ 0.29	3.42 $\pm$ 0.38	5.48 $\pm$ 0.75	6.96 $\pm$ 0.91
Old	Saline	0.85 $\pm$ 0.17	0.95 $\pm$ 0.13	0.89 $\pm$ 0.13	0.72 $\pm$ 0.15	0.86 $\pm$ 0.22
Old	HCG	0.65 $\pm$ 0.15	0.92 $\pm$ 0.08	2.04 $\pm$ 0.42	3.78 $\pm$ 0.44	5.79 $\pm$ 0.71

Figures are ng ml⁻¹, mean $\pm$ s.e. three-month and 8-month-old male rats were cannulated with PE-50 tubing in the right external jugular vein 24 h before initiation of the experiment. 0.25 ml of blood was collected 3–10 min before treatment (control sample). 0.1 IU of HCG per 100 g body weight or an equivalent volume of physiological saline was then injected into the cannula which was rinsed with 0.2 ml of saline. Blood (0.25 ml) was collected 15, 30, 60 and 120 min after injection. Analysis of variance showed a significant increase ($P < 0.005$) in plasma testosterone levels after the injection of HCG. There was no difference in responses between young and old rats. The experiment was run between 1230 and 1600 during August 1974. Animals were purchased from Russell Miller Farms 30 d before the experiment began and maintained in our vivaria in constant conditions as described in the text.

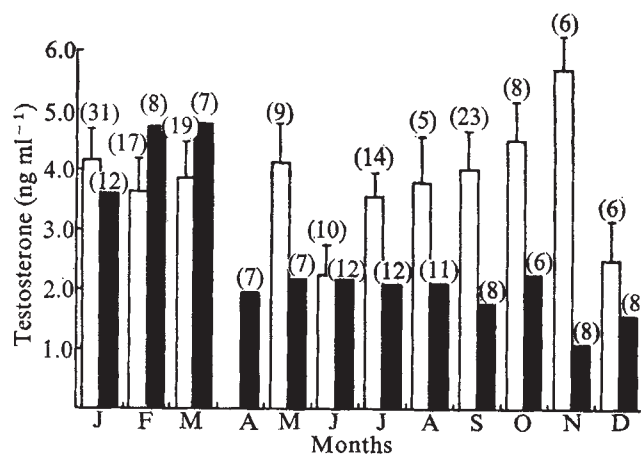


Fig. 3 Comparison of plasma testosterone levels of mature (solid bar) and 60-d-old to 65-d-old (open bar) male rats in different months of the year. The number of animals tested is within the parentheses above each bar. Mean values are graphed; s.e. is included for young animals only. Data of mature rats are from Fig. 1. Rats were bred in our vivaria from the same stock as described in Fig. 1, and reared in constant conditions as described in the text. Analysis of variance showed a significant difference between ages ($P < 0.01$), among months ($P < 0.025$) and a significant age by month interaction ($P < 0.01$).

icular, seasonal fluctuations of plant oestrogens in animal food). It is difficult to sustain this thesis. First, we obtained similar results over several years, using both home-bred and commercially-bred animals which grew up in different environments with different food; second, animals of different birth dates and ages but living in the same environment responded differently to seasonal influences; third, close examination of two recent reports^{14,15} indicates that female rats are also seasonally regulated; and fourth, our animal food purchases and storage were unscheduled.

This report provides the first experimental backing to a growing realisation among scientists that experimental results may differ, depending on the season of the year, particularly when hormonal events with circadian rhythmicity are being examined¹⁶. While the concept that seasonality may be based on internal factors and not on changing environmental conditions is difficult to accept, it now has experimental as well as ideological support^{17,18}.

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An approach to amino acid sequence analysis of transplantation antigens

THE major histocompatibility complex of the mouse, known as the H-2 locus, represents the prime barrier against successful tissue transplants¹ and controls the expression of many cell surface alloantigens which are serologically detectable^{2,3}. It may be subdivided by recombinational analysis into four major regions known as K, D, Ir and Ss (ref. 4). The Ss region controls the quantitative expression of a serum protein⁵. The Ir region controls immune responsiveness of mice to a variety of synthetic and natural antigens^{6,7}, and the expression of multiple cell surface proteins, some of which may be distinguished by molecular weight^{8,9}. The K and D regions both control the expression of distinct cell surface glycoproteins (molecular weight 47,000), which are non-covalently associated with a small molecular weight (11,500) component¹⁰⁻¹¹. Because the native molecules are hydrophobic proteins present on the cell surfaces in small quantities, it is necessary to use unusual procedures for their isolation and chemical characterisation. We have, therefore, used indirect immunoprecipitation and sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) for the isolation and subsequent amino acid sequence analysis of both molecular weight components.

Cell surface proteins of B10.D2 spleen lymphocytes were

Fig. 1 Preparations of the small molecular weight component which had been intrinsically radiolabelled with ³H-tyrosine (Δ) or ³H-lysine (●) were combined and analysed on an automated sequencer. The PTH-amino acid derivative from each cycle was combined with an appropriate mixture of unlabelled PTH-amino acids and separated by one dimensional thin layer chromatography on silica gel plates (Eastman-Kodak) using benzene-acetic acid (9:1) as the chromatographic solvent. PTH-amino acid spots were visualised by short-wave ultraviolet light, excised from the plate, and counted in a liquid scintillation counter. The amount of radioactivity associated with each of the PTH-amino acids is plotted against the step number.

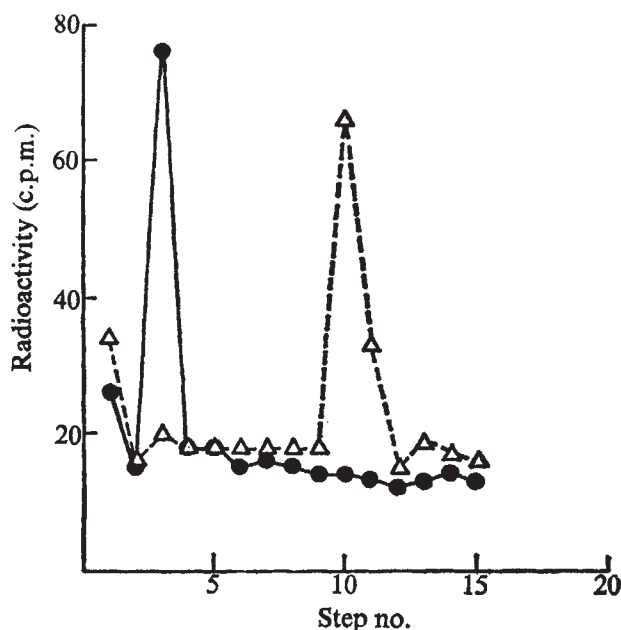


Table 1 Amino acid sequences of B₂-microglobulin

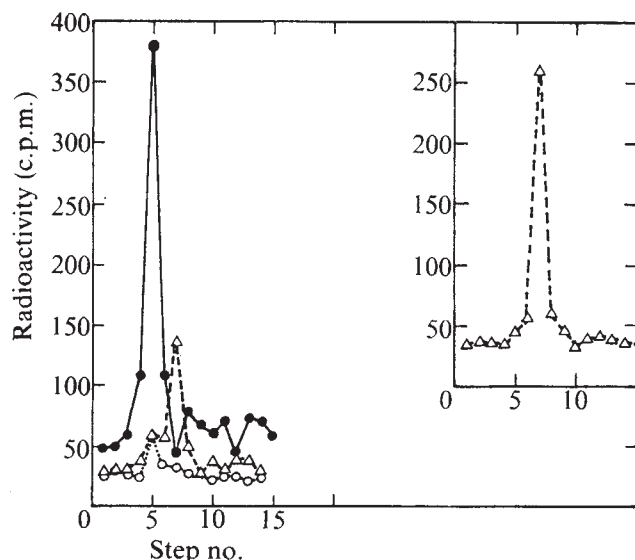
	1	2	3	4	5	6	7	8	9	10
Human	Ile	Gln	Arg	Thr	Pro	Lys	Ile	Gln	Val	Tyr
Dog	Val	Gln	His	Pro	Pro	Lys	Ile	Gln	Val	Tyr
Rabbit	Val	Gln	Arg	Ala	Pro	Asn	Val	Gln	Val	Tyr
H-2 associated polypeptide	—	—	Lys	—	—	—	—	—	—	Tyr

radiolabelled by incorporation of single tritiated amino acids. The H-2.4 alloantigen (product of the H-2D region) and its associated polypeptide were isolated by indirect immunoprecipitation using specific H-2 alloantiserum, and purified on SDS PAGE gels as described previously¹¹. Each molecule was eluted from the gel by overnight incubation of appropriate gel slices in 0.01% SDS. When re-electrophoresed on SDS gels, the eluted material migrated as a single molecular weight peak. Before sequence analysis, each sample was dialysed twice against 0.001% SDS. Sequential protein degradation was performed on a Beckman Model 890 sequencer using the procedure of Hermanson¹². Radioactive phenylthiohydantoin (PTH) derivatives were analysed by thin-layer chromatography as described in Fig. 1.

Sequence analysis of the small molecular weight component enabled the assignment of lysine and tyrosine as the amino acid residues to positions 3 and 10 respectively (Fig. 1). A comparison of our sequence assignment with the amino acid sequence of human¹⁴, rabbit¹⁵ and dog¹⁶ B₂-microglobulin isolated from urine (Table 1) supports our previous suggestion¹¹ that the H-2 associated polypeptide is analogous to the B₂-microglobulin-like molecule found associated with detergent solubilised HL-A alloantigens¹³. Recent preliminary data from our laboratory have enabled us to identify valine as the amino acid residue at position 9 in the small molecular weight component. In addition, rat B₂-microglobulin has been shown by other investigators to have a lysine residue at position 3 (ref. 17). Both findings further support this suggestion.

Amino acid sequence analysis of the H-2.4 alloantigen by this procedure enabled the assignment of leucine and tyrosine residues to position 5 and 7 respectively (Fig. 2). The observed low experimental yield (approximately 40% of theoretical) is probably caused by protein 'washout' from the sequenator cup, and loss of exchangeable tritium.

Fig. 2 Amino acid sequence analysis of the H-2.4 alloantigen radiolabelled with ³H-leucine (●), ³H-tyrosine (△), and ³H-threonine (○) was carried out as described in Fig. 1. The results of a second sequenator run of ³H-tyrosine-labelled H-2.4 alloantigen is shown.



Nevertheless our data suggest that the sequence obtained is representative of a major component of the purified material, and that the H-2.4 alloantigen does not have a blocked N terminus.

These sequence data were obtained from protein which had been isolated from two mouse spleens or approximately 0.15 nmol protein, 1/1,000 the amount normally required for automated sequence analysis. Our results suggest that the H-2 alloantigens as well as other membrane proteins may be amenable to this type of sequence analysis.

Determination of the chemical structure of H-2 and other histocompatibility alloantigens as well as the elucidation of the chemical basis of their antigenicity has implications for tissue and organ transplantation. For example, use of such knowledge may make it possible to render prospective organ recipients specifically tolerant to the unique histocompatibility alloantigens of the prospective donor. In addition, further chemical analysis may clarify the function of the histocompatibility alloantigens and contribute to our basic understanding of cell membrane proteins.

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Stable large variant of 5S RNA in *Clostridium thermosaccharolyticum*

THE precursor forms of prokaryote 16S and 23S ribosomal RNA (rRNA) are larger than their mature counterparts by some 10% (refs 1-5). An analogous large precursor form of the bacterial 5S RNA exists (in at least some cases)^{1,3,6,7}. The reason for the existence of these rRNA precursor forms is not known. They could merely reflect an aspect of the transcription process, or they could have evolved specifically to facilitate ribosome assembly. A more intriguing possibility is that they represent vestiges of ancestral rRNAs, retained because they are essential to ribosome assembly—which process must reasonably recapitulate, to some extent, ribosomal evolution. This last alternative gains some support from our

discovery of a stable form of the 5S RNA some 30–40% larger than normal, in *Clostridium thermosaccharolyticum*.

Polyacrylamide gel profiles of ^{32}P -labelled RNA from *C. thermosaccharolyticum* exhibit an extra band that corresponds to an RNA species of about 160 nucleotides. The new species plus the normal 5S RNA together exist in approximate unit mole ratio to the 16S and 23S rRNAs. The new species is indeed RNA and stable because a high specific activity pulse of ^3H -uridine (>90% incorporated in less than one cell generation) is incorporated into it; and the ratio of ^3H in the new RNA species to that in 5S RNA remains constant (about unity) for at least four generations (the length of the experiment).

Figure 1 is a polyacrylamide gel profile of RNA extracted from the 50S subunit. Both normal 5S RNA and the new RNA species are found on this subunit. Although all this evidence may suggest the new RNA species to be of the 5S RNA genre, definitive proof depends on some more detailed characterisation, such as oligonucleotide fingerprinting.

Figure 2 shows tracings of the two-dimensional electrophoretograms of T1 ribonuclease digests of both the normal 5S RNA (form I) and the new species (form II) from *C. thermosaccharolyticum*. Table 1 catalogues the oligomers found in both T1 and pancreatic ribonuclease digest of the two forms.

Form II contains (almost) every oligonucleotide found in form I. We conclude, therefore, that form II is a '5S' RNA. Form II also has an additional forty or so nucleotides not found in form I, giving the former an approximate size of 160 nucleotides. Both forms contain the same 5' hexanucleotide, pUUUCCG. The 3' terminal oligonucleotide of the shorter form I, however, is absent in form II. The simplest interpretation of this is that the excess material unique to form II occurs as an extension of the 3' terminus of the normal molecule. The presence of several potential stable base-pairing sequences—GGGGU to $[\text{C}_{4-5}\text{U}]\text{G}$ and ACUUUUUUG to GAAAAGU—predicts that the excess material in form II will possess considerable secondary structure.

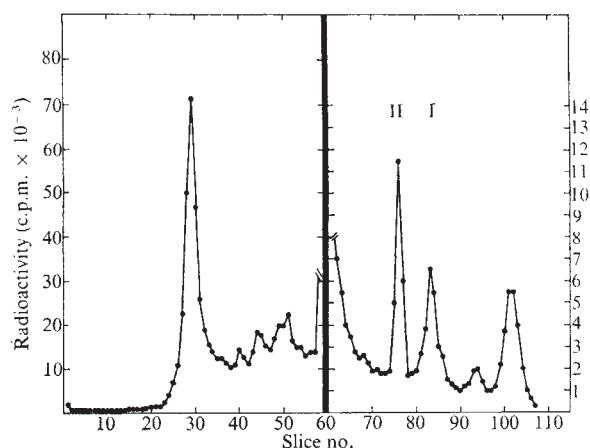


Fig. 1 Polyacrylamide gel electrophoretic profile of RNA from 50S subunit of *C. thermosaccharolyticum*, grown anaerobically in 5 ml dephosphorylated yeast extract-peptone medium to which Na_2S and cysteine were added⁸. Approximately 100 μCi of $^{32}\text{PO}_4$ were added to the culture early in log phase and the culture collected three to four generations later, by low speed centrifugation (12,000g for about 10 min). Cells were ruptured by passage through a French press, and the lysate, cleared of all debris—again by low speed centrifugation—was layered on a 5–20% sucrose gradient in a 10 mM Tris buffer, pH 7.4, containing 0.1 mM MgCl_2 . After centrifugation (90,000g for 9 h in a Spinco SW 25.1 rotor), the resulting 50S peak was collected, dialysed to remove sucrose, and the RNA extracted by the phenol method⁹, the purified RNA being subsequently analysed by polyacrylamide gel electrophoresis. A split gel method was used¹⁰, the initial portion comprising 3.2% acrylamide (a), the final portion, 10% acrylamide (b). The large peak in the 3.2% gel section has the mobility one would expect for 23S rRNA; the peaks labelled I and II in the 10% section of the gel have the mobilities expected for normal 5S RNA and the new RNA species, respectively. The remaining material in the 10% section of the gel is presumably tRNA and/or 23S rRNA breakdown products.

Table 1 Sequences and molar occurrence of oligomers (dimer and larger) produced by T1 or pancreatic ribonuclease digestions of forms I and II of *C. thermosaccharolyticum* 5S RNA

Oligomer	T1 ribonuclease		Oligomer	Pancreatic ribonuclease	
	I	II		I	II
CG	2 1/2	2 1/2	AC	4	4
AG	4	3 1/2	AU	1 1/2	2 1/2
UG	2 1/2	3 1/2	GC	3	4 1/2
			GU	1 1/2	2
CCG	2	2	AGC	2	2 1/2
AAG	2 1/2	1	GAU	2	3
UCG	1	1 1/2	AGU	0	1
CUG	1	1	GGC	1	1
UAG	1	1	GGU	2	4
AUG	1	1	AAU	1	1
CAAG	0	1	GAAC	1	1
AUAG	0	1	AAAU	0	1
AAAAG	0	1	GAGU	1/2	0
UACUG	1	1	AGGU	1	1
AACACG	1	1	AAGAC	0	1
CAAUAG	1	1	GAAGC	1	1
$[\text{C}_{4-5}\text{U}]\text{G}$	0	1	GGGU	0	1
CCC(CU)CAG	1	1	AAAAAC	1	1
AUUACUCG	1	1	GGAAGU	1	0
ACUUUUUUG	0	1	GG[G, AG]U	1	1
(UCCC)AAAUCG	0	1	GAAAAGU	0	1/2
UUAAAAACCCG	1	1	GGGAGAGU	1	1
U(UC)CCAUCCG	1	1	pU	1	1
pUUUCCG	1	1	(AG) C_{OH}^*	0	1
AUAAU OH	1/2	0			

Oligomer spots shown in Fig. 2 and comparable spots from pancreatic nuclease fingerprints were sequenced as described previously¹²⁻¹⁴. Where order is uncertain in an oligomer, the nucleotides in question are in parentheses. Quantification by scintillation counting of excised spots in a non-aqueous scintillant (BBOT) are given to the nearest 0.5 mol ratio.

*Identification not certain.

A possible explanation for form II is that it is related, at least in an evolutionary sense, to 5S precursor forms^{6,7}. Sogin and Pace have shown that the conversion from precursor to

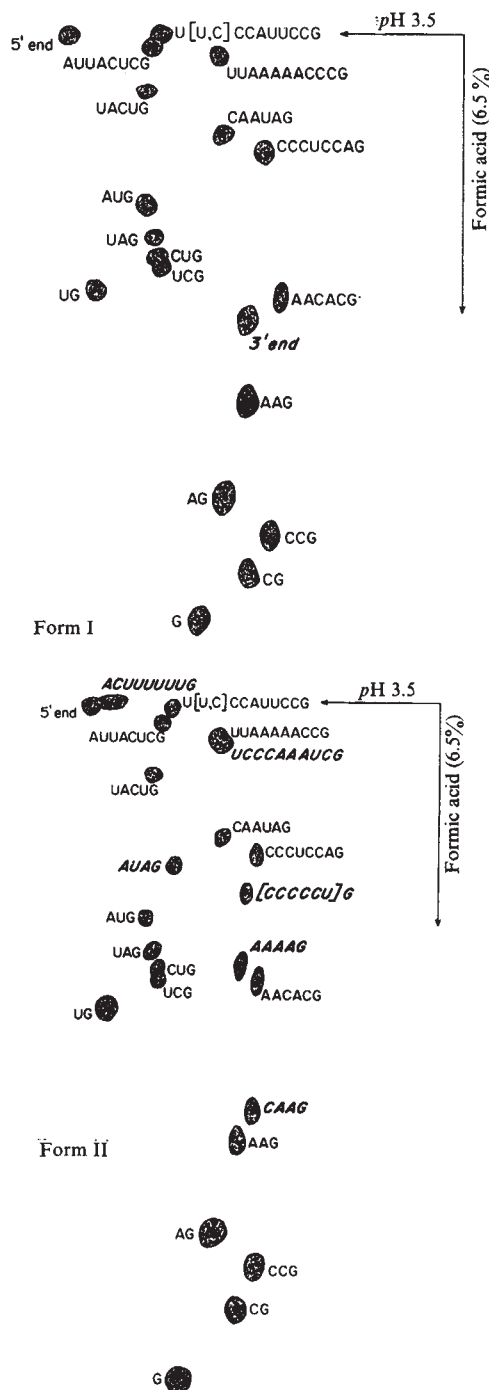


Fig. 2 Tracings of two-dimensional electrophoretograms of T1 ribonuclease digests of 5S RNA (form I) and the new RNA species (form II). *C. thermosaccharolyticum* was grown, labelled, and cells ruptured as described in Fig. 1, except that 5–10 mCi ³²PO₄ were added to the culture. Cell lysate was extracted immediately by the phenol method⁹, and the resulting RNAs isolated by electrophoresis on 10% polyacrylamide gels. Appropriate RNA bands were located by radioautography of the intact gel, the bands cut out, and the RNA extracted by phenol¹¹. Specific activities of the RNA varied from preparation to preparation, but were within the range 0.5–2 μ Ci per μ g RNA. The two RNAs were purified by passage over BD-cellulose¹⁰, digested with T1 ribonuclease and the resulting products analysed by a modification of two-dimensional electrophoretic method^{12–14}. The first electrophoretic dimension was run on cellulose acetate at pH 3.5, the second on DEAE-cellulose paper in a 0.1 M pyridine solution brought to pH 2.3 with formic acid (ref. 14 and M. Sogin, D. Stahl, L. Bonen and C.W., unpublished). Oligonucleotides unique to Form II are italicised.

mature 5S RNA (in Bacilli) involves cleavage by the same enzyme at points defining both the 5' and 3' termini of the mature 5S RNA (ref. 7). An identical large purine stretch is found about 20 nucleotides proximal to the point of cleavage in each instance, and presumably therefore, serves as a recognition signal for the enzyme, RNase m5 (ref. 7). Were this the general mechanism for 5S RNA maturation (in prokaryotes), it would demand in the present case that form II not contain such a signal defining a normal 3'-terminal cleavage point. Indeed, form II does not possess one large purine stretch, GGAAGU, found in form I, but its position in the molecule is unknown at present.

The existence of a large form of the 5S RNA raises many questions regarding both function and evolution in the ribosome. The trivial explanation that the large form of 5S RNA has neither functional nor evolutionary significance cannot be ruled out, but it seems unlikely. Ratios of the two forms of 5S RNA from cells grown at the extremes of the organisms' growth range (that is, 45 and 65 °C) are not significantly different, ruling out the possibility that one form may be used preferentially at higher growth temperatures. We have not determined whether this ratio varies during sporulation.

As yet, the stable large form of 5S RNA is unique to *C. thermosaccharolyticum*. It is definitely not present in Bacilli^{6,7}, and we have looked for, but failed to find it in mesophilic Clostridia, even in representatives of the same subgroup classification as *C. thermosaccharolyticum* (K. Luehrsen and C.W., unpublished).

Two explanations remain. It could be a retention of or return to an ancestral type of 5S RNA—the latter being made possible through retention of ancestral characteristics in precursor forms of the 5S molecule. On the other hand, it may represent a novel evolutionary happening, having no resemblance to archaic forms of 5S RNA. We do not favour the latter on the grounds that the change in passing from the normal to enlarged form of 5S RNA is a drastic one (and would probably require further evolutionary changes in the ribosome), and it would seem *a priori* unlikely to occur in a fully evolved ribosome (that is, in one species of one genus).

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Oxygen binding to haemoglobins of the primitive vertebrate *Myxine glutinosa* L.

THE Atlantic hagfish (*Myxine glutinosa* L.) belongs to the family Myxinidae which forms along with the family Petromyzonidae (the lampreys) the only living class of jaw-

less vertebrates (Agnatha), the Cyclostomata. These Cyclostomata are considered on morphological¹ and physiological^{2,4} evidence to be the most primitive form of living vertebrates and are distant descendants of fossil forms of Agnatha which were already present during the Ordovician, that is, more than 400 Myr ago^{1,3}.

The oxygen-binding characteristics of hagfish haemoglobin offer an opportunity to obtain information on the evolution of the respiratory function of haemoglobin. It seems⁴⁻¹⁰ that hagfish haemoglobin, unlike all known haemoglobins of higher vertebrates which carry four haems per molecule, has only one oxygen-binding site. Not unexpectedly they exhibit little, if any, allosteric effects, such as cooperativity of oxygen binding and Bohr effect, and would be therefore called 'primitive' in comparison to more advanced forms of molecular organisation of respiratory proteins represented by tetrameric haemoglobin. If the oxygen-binding characteristics of the haemoglobins of *M. glutinosa* reported here, however, are compared with those of the Pacific^{3,6} (*Eptatretus stoutii*) and Japanese⁷ (*E. burgeri*) hagfish, it seems that these groups of animals form distinct functional entities and can be called 'primitive' only from a comparative point of view.

Preparation of haemoglobin solutions from the blood of *M. glutinosa* and fractionation of the three major components (Hb_I, Hb_{II}, Hb_{III}) was carried out as described previously^{9,10}. We found that the three major types of haemoglobin which are present in the blood of *M. glutinosa* have different oxygen affinities (Fig. 1), but did not show any sign of cooperativity when judged from the exponent *n* in the Hill equation¹³. Even though Hb_I, Hb_{II} and Hb_{III} represent only 52% of the total (the remainder being minor components) they seem to govern the oxygen affinity of the total haemolysate. This can be deduced from the good agreement between the partial pressure of oxygen at which haemoglobin is half saturated with oxygen (P_{50}) measured in the total haemolysate (4.2 mmHg at pH 7.3 and 20 °C) and that calculated from the respective percentage of each component⁹ (4.3 mmHg in identical conditions). Hb_{II} and Hb_{III} did not change oxygen affinity in the pH range between 6.85 and 8.20, that is, they did not exhibit the Bohr effect. Note that multiple haemoglobin fractions of *E. burgeri* also showed functional heterogeneity⁷ although the major compounds A, B and C found in *E. stoutii* did not⁶. Thus, there is haemoglobin polymorphism in all three species of hagfish but in only two of them do different molecules

Fig. 1 Plot of $\log(y/(1-y))$ against $\log P_{O_2}$ for the isolated haemoglobins Hb_I (○), Hb_{II} (△), Hb_{III} (□) of hagfish blood (Hill plot¹³). *Y* represents saturation of haemoglobin with oxygen. P_{50} : 3.7, 2.3 and 6.3 mmHg for Hb_I, Hb_{II} and Hb_{III} respectively. Hill's exponent *n* averaged 1.03 for the three components. Temperature was 20 °C, pH 7.3 and haemoglobin concentration 0.1×10^{-3} M l⁻¹.

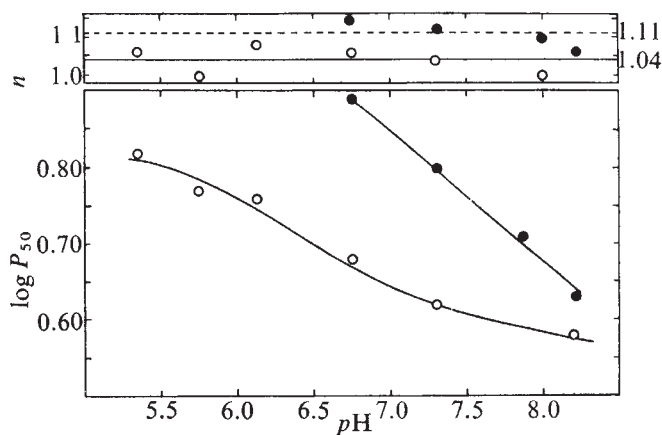
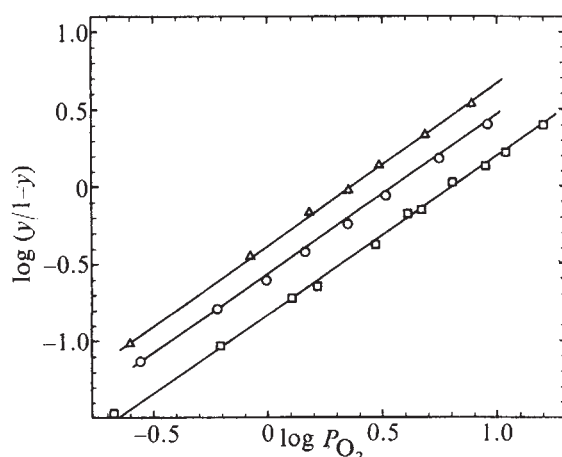


Fig. 2 Plot of $\log P_{50}$ against pH of hagfish haemolysate. ○, Without CO₂; ●, in presence of CO₂. Note that in these latter experiments pH was changed by varying P_{CO_2} at constant (23.9×10^{-3} M l⁻¹) bicarbonate concentration. Upper part of figure shows the exponent *n* of the Hill equation¹³ in which the lines represent the means obtained in the absence and presence (dotted line) of CO₂. Temperature was 20 °C and haemoglobin concentration 0.1×10^{-3} M l⁻¹. Buffers were 50 mM bis-Tris-100 mM NaCl in the absence of CO₂ and bicarbonate-NaCl buffer with a molarity of 150 mEq l⁻¹ in the presence of CO₂. As methaemoglobin was present in some preparations, the samples were reduced with sodium dithionite and the reaction products removed on a mixed-bed ion-exchange column¹¹ before oxygen-binding experiments¹².

have different oxygen affinities. In particular environmental conditions such differences in haemoglobin function could be of physiological significance. If, for example, ambient temperature is raised, an increased synthesis of Hb_{II} which has a high oxygen affinity (Fig. 1) would counteract the impaired oxygen uptake of the animal brought about by the high heat of oxygenation (-12.7 calorie mol⁻¹) which we determined in the haemolysate of *M. glutinosa*.

Consider the oxygen-binding properties of the whole haemolysate (Fig. 2). In the pH range of physiological importance for hagfish^{4,5} (7.5-7.8) there was very little change in oxygen affinity with varying pH ($\Delta \log P_{50}/\Delta pH = -0.07$) at least in the absence of CO₂, thus confirming the results of Manwell⁴. At pH less than 6.5 we observed a more pronounced Bohr effect which is, however, of no significance for the gas exchange in *M. glutinosa*. Addition of CO₂, on the other hand, significantly decreased oxygen affinity and increased the Bohr effect to -0.17 ($\Delta \log P_{50}/\Delta pH$) in the range between pH 7.3 and 8.2. This relatively important increase in Bohr effect in the presence of CO₂ is still small in absolute terms and therefore compatible with the notion that fish living in an environment with varying CO₂ pressures (as the burrowing hagfish probably does), have in general a reduced Bohr effect^{4,8}. Furthermore, the Hill parameter *n* increased significantly ($0.02 > P > 0.01$) on addition of CO₂, indicating that cooperativity of oxygen binding increased. This is probably caused by an increased tendency of the various components of *M. glutinosa* haemoglobin to aggregate. Such aggregation, also found in *E. burgeri*⁷ and *Petromyzon marinus*^{14,15} at low pH and high haemoglobin concentration, may be interpreted as an early attempt of nature to form the cooperative heterotetramers¹⁶, which carry oxygen in the blood of higher vertebrates. In the isolated components Hb_I, Hb_{II} and Hb_{III} this specific effect of CO₂ on the oxygen-binding properties was not observed. Hb_I and Hb_{II} have blocked N-terminal amino groups, whereas in Hb_{III} the N terminus is free to form carbamino compounds. This binding of CO₂ to Hb_{III} does not seem to lead to self-aggregation which reflects itself in decreased oxygen affinity.

The question arises therefore how the CO₂ effect can be

explained. If it is not mediated by Hb_{III} there must be one or several minor components whose oxygen affinity is considerably decreased by CO₂. As the haemolysate of *M. glutinosa* contains more than ten of these minor fractions^{9,10}, none of which has been structurally characterised, it is impossible at present to identify the ones which may indicate the CO₂ effect. Nevertheless, the possibility should be kept in mind that the decrease in oxygen affinity produced by CO₂ could be brought about by interactions between different haemoglobin molecules. Such intermolecular interactions have a significant influence on the oxygen binding properties in the haemolysate of *E. burgeri*, even though the isolated, homogeneous components show practically no tendency to associate⁷. By the same token, it seems possible that the increased surface charge at specific sites in Hb_{III} (or some minor fractions), which is the result of carbamate formation, leads to an aggregate product between different molecules with a consecutive decrease in oxygen affinity of the whole haemolysate. In contrast to CO₂, other effector molecules which have large effects in tetrameric haemoglobin, like 2,3-diphosphoglycerate, inositol hexaphosphate and high concentrations of NaCl had no influence on oxygen affinity and cooperativity of *M. glutinosa* haemoglobin. Even an increase in protein concentration by a factor of six had no effect on P_{50} or the exponent n , in contrast to what is found in *E. burgeri*⁷.

If we compare the P_{50} values which have been obtained in different species of hagfish in nearly identical conditions with respect to haemoglobin concentration, pH and temperature, *E. burgeri* has the lowest oxygen affinity (P_{50} =10 mmHg)⁷ being followed by *M. glutinosa* (P_{50} =4.2 mmHg). *E. stoutii* has the highest oxygen affinity (P_{50} =1.6 mmHg)^{5,6}. As long as we know as little as we do on ambient conditions and the respiratory capacity of these animals it would be inappropriate to draw any far reaching conclusion on possible ecological advantages of such differences in oxygen-binding characteristics of hagfish haemoglobin. These particular respiratory proteins seem to be primitive in the sense that they exhibit very limited allosteric effects. Note, however, that hagfish are biologically very successful animals so that their haemoglobins are likely to fulfil their physiological function quite well. Oxygen exchange, for example, takes place at such low internal partial pressures of oxygen in these animals⁴ that the absence of significant cooperativity of oxygen binding to haemoglobin does not seem to have any adverse effects on oxygen delivery to the tissues.

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Role of light and rhodopsin phosphorylation in control of permeability of retinal rod outer segment disks to Ca²⁺

It has recently been shown in several laboratories that if isolated rod outer segments (ROS) are incubated in the presence of MgATP, rhodopsin is phosphorylated (see ref. 1). The reaction is much faster after the ROS have been exposed to light as the enzyme, 'opsin kinase', is specific to bleached rather than unbleached rhodopsin¹. It has been suggested that the function of rhodopsin phosphorylation may be to regulate the responsiveness of ROS to light and in this paper we describe a mechanism whereby such a regulation could occur.

It is well established that the absorption of photons by

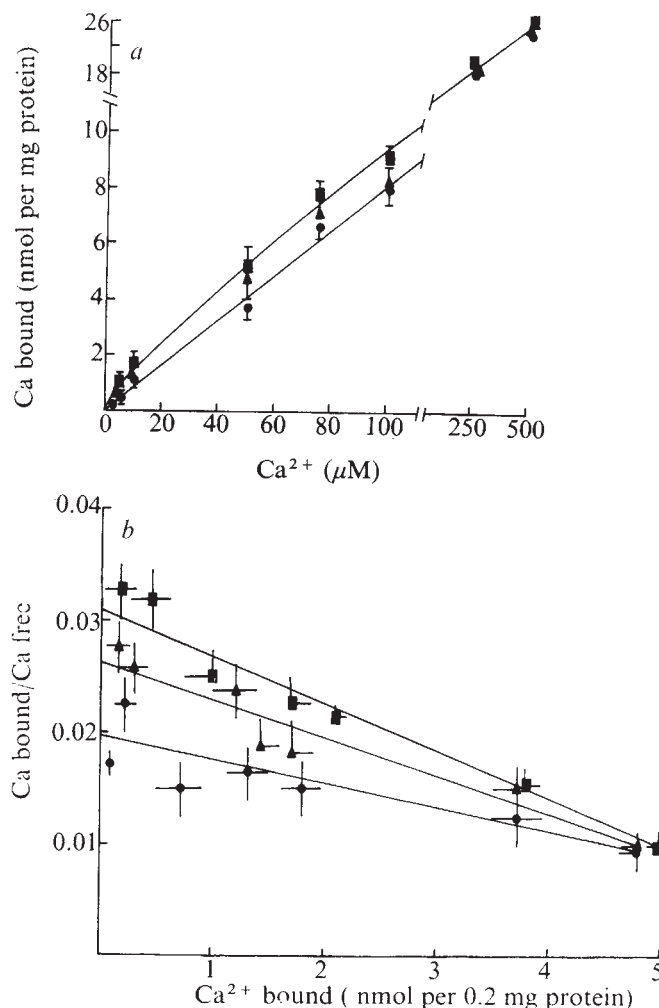


Fig. 1 Effect of Ca²⁺ concentration on the binding to ROS disks. Freshly prepared ROS were either kept in dim red light (●), exposed to white light for 2 min (Δ), or exposed to white light for 2 min and phosphorylated at 37 °C at a protein concentration of about 0.5 mg ml⁻¹ in 50 mM Tris-HCl, pH 7.4, and 1 mM MgATP for 30 min¹ (■). All samples were then washed by centrifugation from 10 mM Tris-HCl, pH 7.4, and incubated in dim red light at 37 °C at a protein concentration of about 0.2 mg ml⁻¹ in 1 ml aliquots of 10 mM Tris-HCl, pH 7.4, and the stated concentration of ⁴⁵CaCl₂ (specific radioactivity about 2 × 10⁷ c.p.m. μmol⁻¹) for 2 h to reach equilibrium (Fig. 3). Samples were then filtered using wet Millipore cellulose ester filters (0.45 μm pore size) and the filters washed with three 5 ml aliquots of 10 mM Tris-HCl, pH 7.4, placed in scintillation vials, and dried at 80 °C before adding scintillation fluid and counting. The washing procedure took less than 2 min and less than 2% of protein passed through the filters. Results are taken from five experiments and are shown as means ± s.d.

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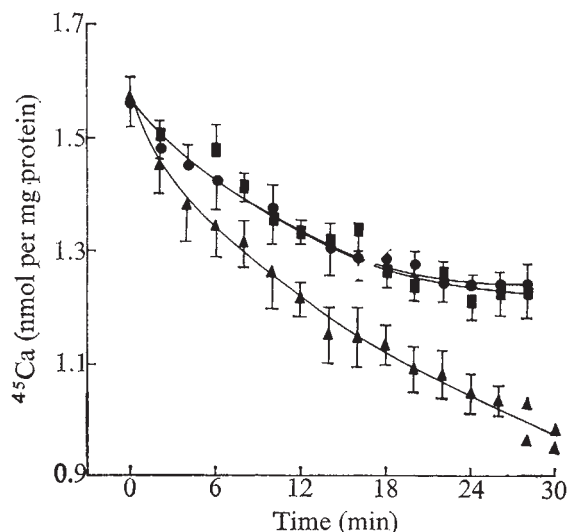


Fig. 2 Effect of exposure to light and phosphorylation on the rate of loss of Ca^{2+} from ROS disks. Freshly prepared ROS were either kept in dim red light (●), exposed to white light (▲), or exposed to white light and phosphorylated (■), and all samples washed with 10 mM Tris-HCl as described in Fig. 1. Samples were then incubated with $10 \mu\text{M}$ $^{45}\text{CaCl}_2$ (specific radioactivity 2×10^7 c.p.m. μmol^{-1}) in 0.3 M sucrose and 10 mM Tris-HCl, pH 7.4, at a protein concentration of about 0.3 mg ml^{-1} for 2 h and aliquots taken to determine the concentration of bound $^{45}\text{Ca}^{2+}$. The remaining material was centrifuged at 10^5g for 15 min and the pellets suspended in 10 mM Tris-HCl, pH 7.4, containing 0.3 M sucrose at a protein concentration of about 0.3 mg ml^{-1} . Samples (0.5 ml) were then taken at stated times and filtered through Millipore filters to determine the amount of $^{45}\text{Ca}^{2+}$ bound as described in Fig. 1. The time between the start of resuspension of the loaded pellets and the taking of the first sample was always 3 min. Results are taken from six experiments and show means $\pm$ s.d. The concentration of bound $^{45}\text{Ca}^{2+}$ at the end of the incubation with $^{45}\text{CaCl}_2$ was 1.78 ± 0.16 , 1.90 ± 0.1 and $1.93 \pm 0.1 \text{ nmol, } ^{45}\text{Ca}^{2+} \text{ per mg protein}$ for dark-kept, light-exposed, and light-exposed phosphorylated material, respectively.

vertebrate rods causes a hyperpolarisation which is thought to be the result of a decrease in Na^+ permeability of the ROS (refs 2–4). It is this hyperpolarisation which enables the photochemical apparatus of the outer segment to transmit its state as a nervous impulse. It is, however, difficult to see how the absorption of a single photon by the plasma membrane could cause a reduction in dark current by as much as 3% (ref. 5). Moreover most, if not all, of the light absorbing molecule, rhodopsin, is located not in the plasma membrane of the ROS but in the intracellular disks which are not connected directly to the outer plasma membrane^{5,6}. These observations suggest that a transmitter is released from the ROS disks which interacts with the outer plasma membrane lowering its permeability to Na^+ (refs 3 and 7). Hagins^{3,7} has suggested that the transmitter may be Ca^{2+} as treatment of ROS with Ca^{2+} causes their hyperpolarisation^{7,8}, and isolated ROS disks show an active uptake of Ca^{2+} (ref. 9). A light-induced efflux of calcium from ROS disks has also been described^{10–13}. These observations indicate that even if Ca^{2+} is not the actual transmitter, it will have an important role in modulating the transmission of light as a nervous impulse.

There have been several reports that phosphorylation of plasma membrane proteins from liver¹⁴, heart^{15,16} or skeletal muscle¹⁷ can increase the binding of Ca^{2+} to these membrane fragments. For this reason we investigated the effect of phosphorylation of rhodopsin on the Ca^{2+} -binding and permeability properties of ROS disks.

ROS were prepared from calf eyes as described previously¹⁸ and disk membranes and membrane fragments obtained by centrifugation at 10^5g for 60 min after rupturing the ROS outer

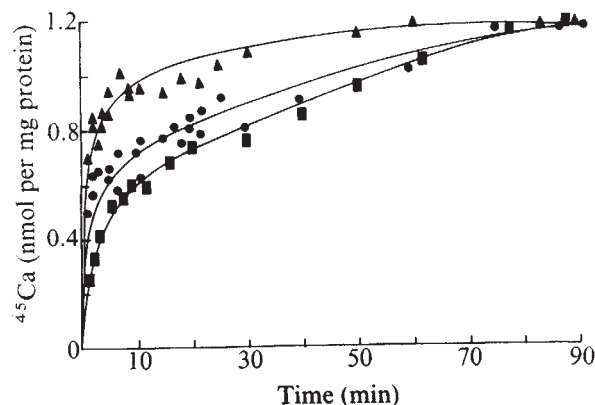
membrane by homogenisation in 10 mM Tris-HCl, pH 7.4 (ref. 19). The binding of Ca^{2+} to the disk membranes was determined after incubating to equilibrium as described in Fig. 1, from which it may be seen that there is little difference between membranes of ROS disks that have been kept in the dark, exposed to light or exposed to light and phosphorylated; although exposure to light and, in particular, phosphorylation of the light-exposed material, did cause some slight increase in Ca^{2+} binding. It has recently been reported that the amount of Ca^{2+} bound to ROS was greatly increased on exposure to light¹². This result was probably obtained because incubations were not carried out for sufficient time to reach equilibrium (Fig. 3).

We next examined the effect of light on the rate of Ca^{2+} loss from ROS disks. Figure 2 shows that if the disk membranes were exposed to light before loading with $^{45}\text{Ca}^{2+}$ the subsequent efflux of radioactivity on suspension in Ca^{2+} -free medium was greater than that observed if the material was kept in the dark. The effect was prevented if the light-exposed material was phosphorylated by incubation with MgATP. Incubation with ATP in the absence of Mg^{2+} gave no effect. A similar effect of light was found whether the ROS disks were illuminated before or after loading with $^{45}\text{Ca}^{2+}$. As exposure to light causes, if anything, a slight increase in Ca^{2+} binding (Fig. 1) the increased $^{45}\text{Ca}^{2+}$ loss on exposure to light can scarcely be the result of an effect on binding; similarly phosphorylation causes such a small change in binding to light-exposed material that it is probably not important in affecting the rate of ^{45}Ca loss. It seems probable that Ca^{2+} entered the disks during incubation with $^{45}\text{Ca}^{2+}$ and that exposure to light increases calcium loss on suspension in Ca^{2+} -free media because of an increase in permeability of the disk membranes which makes it easier for Ca^{2+} to leave the loaded saccules, whereas phosphorylation of the light-exposed material lowers the membrane permeability.

If ROS disks which have been exposed to light are indeed more permeable to Ca^{2+} then the rate of entry of Ca^{2+} should also be increased. This was, in fact, found to be the case (Fig. 3); if the light-exposed material is phosphorylated then the rate of Ca^{2+} entry is lowered to a little less than that observed in material kept in the dark. Results shown in Figs 2 and 3 are thus comparable.

Reports have shown a decrease in bound Ca^{2+} on exposure of ROS to light^{10–13}, but it was not possible from those experiments to know if the result was the result of a reduction in

Fig. 3 Effect of light and phosphorylation on the rates of entry of Ca^{2+} into ROS disks. Freshly prepared ROS was either kept in dim red light (●), exposed to white light (▲), or exposed to white light and phosphorylated (■), and all samples washed with 10 mM Tris-HCl, pH 7.4, as described in Fig. 1. Disks were then suspended at a protein concentration of about 0.3 mg ml^{-1} in 10 mM Tris-HCl, pH 7.4, containing 0.3 M sucrose and $10 \mu\text{M}$ $^{45}\text{CaCl}_2$ (specific radioactivity 2×10^7 c.p.m. μmol^{-1}). Samples (0.5 ml) were taken at stated times and filtered to determine the amount of bound $^{45}\text{Ca}^{2+}$ as described in Fig. 1. Similar results have now been obtained in four separate experiments.



Ca^{2+} -binding sites or of a change in permeability. Our results clearly demonstrate that a change in permeability occurs.

If one accepts Hagin's hypothesis that a nervous impulse is triggered from ROS by the release of Ca^{2+} from the interdiskal space, then the observation that phosphorylation of bleached rhodopsin in the disks blocks the release is one of considerable importance. When light reaches the outer segments of the retina in the intact eye rhodopsin molecules will be bleached and a release of Ca^{2+} from the disks will certainly result. Within a fairly short time, however, bleached rhodopsin molecules will be phosphorylated and as this will lower Ca^{2+} permeability of the disks, they will be able to pump back Ca^{2+} from the extradiskal space. Such a mechanism would have considerable importance in the adaptation of retinal sensitivity to background light conditions. It is well known that the eye becomes progressively less sensitive with increasing background illumination; it seems likely that the fact that in such conditions increasing numbers of receptors are 'switched off' by phosphorylation will be of considerable importance in this phenomenon. On exposure to dark conditions the bleached rhodopsin will be regenerated and dephosphorylated so that the light receptors will be 'switched on' again, a fact which is likely to have considerable importance in dark adaptation. The time course of the loss of phosphate from phosphorylated rhodopsin in the intact frog retina is indeed the same as the time course of dark adaptation²⁰.

Plasma membrane fragments from many tissues, including brain²¹⁻²³, heart muscle^{23,24}, kidney²³ and liver²³, contain tightly bound kinase enzymes responsible for the phosphorylation of the membrane proteins. In these cases cyclic AMP stimulates the enzyme and has also been shown to cause an increase in efflux of Ca^{2+} from the kidney collecting tubule^{25,26} or liver slice^{27,28}, and an increase of Ca^{2+} uptake into rat heart microsomal fragments^{29,30}. Several workers have shown that phosphorylation of membrane proteins from liver¹⁴, skeletal muscle¹⁷ or heart muscle^{15,16} causes an increase in Ca^{2+} binding, but no attempts have been made to measure possible changes in Ca^{2+} permeability. In the case of toad bladder, however, cyclic AMP causes an increase in the efflux of $^{45}\text{Ca}^{2+}$ presumably as a result of an increase in permeability^{26,31}. In this tissue cyclic AMP stimulates the loss of phosphate from plasma membrane proteins, thus lowering the concentration of protein-bound phosphate³². Thus, in the toad bladder cell membrane, a decrease in protein-bound phosphate is associated with an increase in permeability to Ca^{2+} , whereas in ROS disks an increase in protein phosphorylation is associated with a decrease in permeability to Ca^{2+} .

It may be seen therefore that in the case of membranes of both ROS disks and toad bladder increased protein phosphorylation is associated with a decrease in permeability to Ca^{2+} . We suggest that changes in the state of protein phosphorylation have a general role in regulating Ca^{2+} permeability and/or binding in the cell membrane.

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Divalent cation ionophore A23187 forms lipid soluble complexes with leucine and other amino acids

THE *Streptomyces* antibiotic A23187, a divalent cation ionophore¹, has been used as a tool in studies of the role of calcium in several cellular phenomena²⁻⁵. A23187 has a much higher affinity for divalent than monovalent cations, and the increased permeability induced by this ionophore in cellular membranes favours calcium and magnesium. X537A, another divalent cation ionophore⁶, also enhances fluxes of monovalent cations⁷ and forms lipid-soluble complexes with norepinephrine and some other organic compounds⁸. A23187 has often been referred to as a specific divalent cation ionophore although, to our knowledge, the evidence for the specificity is restricted to the lack of interaction with common monovalent cations like sodium and potassium^{1,2}. In this paper we report experiments which show that A23187 is able to form lipid-soluble complexes with leucine and other amino acids at concentrations as low as or lower than those needed to form similar complexes with calcium.

We have been studying the mitogenic effect of A23187 on human peripheral blood lymphocytes in culture (submitted for publication). During these studies we found that A23187, like the lectin mitogens⁹, caused an enhancement of the rate of amino acid uptake by the cells within 3 h of culture. Unexpectedly, ethyleneglycol-bis-(aminoethylether)-tetra-acetic acid (EGTA) in concentrations which entirely blocked calcium uptake did not abolish the increased rate of amino acid uptake induced by the ionophore. We therefore examined possible direct interactions of the ionophore with leucine.

First, we investigated the effect of A23187 on the partitioning of L-leucine between an aqueous and an organic phase. ³H-leucine and 10 μM A23187 in 1 ml phosphate-buffered saline (PBS) were shaken vigorously with the same volume of toluene-butanol (1:1 or 7:3 v/v) mixture^{2,10}. The ionophore was able to increase the proportion of ³H-leucine recovered in the organic phase by 5 to 20-fold (Table 1).

In preliminary experiments we found that some organophilic impurity was present in the ³H-leucine preparation. Therefore, in later studies the ³H-leucine solution was extracted once with the toluene-butanol mixture before using it in the

Table 1 Organic sequestration of ^3H -leucine by A23187

Composition of the initial water phase	Amount of leucine in the organic phase after extraction	
	% c.p.m.	nM
Experiment 1		
Leucine (18.5 nM)	2.34 $\pm$ 0.02	0.43
Leucine (18.5 nM)+A23187 (10 μM)	11.87 $\pm$ 0.25	2.19
Leucine (18.5 nM), pre-extracted	1.56 $\pm$ 0.01	0.29
Leucine (18.5 nM), pre-extracted+A23187 (10 μM)	12.04 $\pm$ 0.31	2.23
Experiment 2		
Cycle I:		
(A) Leucine (18.5 nM)	1.09 $\pm$ 0.01	0.20
(B) Leucine (18.5 nM)+A23187 (10 μM)	19.76 $\pm$ 1.85	3.65
(C) Leucine (18.5 nM)+ethanol (1 %)	1.10 $\pm$ 0.01	0.20
Cycle II:		
PBS only, shaken with the organic phase resulting from:		
Cycle IA	18.30 $\pm$ 0.40	0.04
Cycle IB	23.55 $\pm$ 0.25	0.86

^3H -L-Leucine (1 μCi ; 18.5 pmol); (Radiochemical Centre, Amersham, UK) in 1 ml PBS without divalent cations $\pm$ the ionophore as indicated was shaken with 1 ml toluene-butanol mixture for 1 min at room temperature in a conical glass centrifuge tube. Phase separation was facilitated by brief centrifugation. Aliquots of both the organic and the water phase were counted for radioactivity in identical conditions in a toluene-based scintillation cocktail. Total recovery of counts was more than 97 %. Mean and range of the results from duplicate extractions are given. Approximate nM concentration of leucine in the final organic phase is calculated from the c.p.m. assuming that 100 % of the radioactivity represents leucine.

Experiment 1: pre-extracted = ^3H -leucine stock solution extracted once with toluene-butanol before use in the actual experiment. Toluene-butanol ratio 1:1 v/v.

Experiment 2: Toluene-butanol ratio 7:3 v/v. % c.p.m. in cycle II is calculated with reference to the total amount of radioactivity in cycle II.

actual experiment. This procedure did not affect the A23187-induced sequestration of ^3H -leucine (Table 1, experiment 1). As the 10 mM stock solution of the ionophore was made up in ethanol, we included 1 % ethanol in our controls. No significant increase in the proportion of ^3H -leucine in the organic phase was seen (Table 1, experiment 2). The A23187-induced sequestration of leucine was to a great extent reversible, as shown by re-extracting the organic phase with PBS (Table 1, experiment 2). As the vast majority of the ionophore is retained in the organic phase in these conditions¹⁰, this finding suggests that some of the lipid-soluble complexes formed by leucine and the ionophore are dissociated during re-extraction with an aqueous phase. That the sequestration of ^3H -label by A23187 really represents transfer of leucine to the organic phase, and not that of a hypothetical radioactive impurity, was finally shown by displacing ^3H -label from the organic phase by unlabelled leucine (Table 2). These experiments also demonstrated that the molar ratio in the complexes formed by leucine and A23187 may be as high as 1:2, which is the figure reported for the divalent cation-A23187 complexes^{2,10}. Ionophore (10 μM) was able to increase the concentration of leucine in the organic phase up to 6 μM (Table 2). Direct

measurements of leucine and ionophore concentrations are, however, needed to establish this ratio. Compared with cold leucine, CaCl_2 was clearly less effective in inhibiting sequestration of ^3H -leucine (Table 2). On the other hand, leucine seemed to be practically unable to affect the transfer of ^{45}Ca to the organic phase (Table 2). These findings could be explained by assuming that leucine and calcium are bound to different sites in the ionophore molecule, or that the $(\text{A23187})_2\text{-Ca}$ complex is not accessible to leucine, whereas complexes formed by the ionophore and leucine are sensitive to calcium ions. These competition experiments were complicated by the finding that 100 μM A23187, which was the concentration necessary to obtain significant sequestration of ^{45}Ca , was practically unable to transfer leucine to the organic phase, whereas such transfer was marked with the 10 μM concentration of the ionophore (Fig. 1). Almost identical results were obtained in three separate experiments. Higher concentration of ethanol in the 100 μM A23187 system was not the reason for the difference between the effects of 10 μM and 100 μM ionophore, as the presence of the same concentration of ethanol in the 10 μM system had no effect on sequestration of leucine or calcium (data not shown). We do not know the reason for the failure of 100 μM

Table 2 Competition between leucine and calcium for A23187

Composition of the initial water phase	% c.p.m. recovered in the organic phase	Organic sequestration % Inhibition	Calculated μM concentration of leucine or Ca in the final organic phase
^3H -leucine (0.0185 μM)+A23187 (10 μM)			
+ PBS	8.90 $\pm$ 0.32	—	0.0016
+ L-leucine (50 μM)	3.13 $\pm$ 0.64	65	1.57
+ L-leucine (500 μM)	1.22 $\pm$ 0.06	86	6.10
+ CaCl_2 (50 μM)	7.30 $\pm$ 1.13	18	0.0013
+ CaCl_2 (500 μM)	4.30 $\pm$ 1.04	52	0.0008
$^{45}\text{CaCl}_2$ (0.80 μM)+A23187 (100 μM)			
+ PBS	14.7 $\pm$ 0.9	—	0.12
+ L-leucine (50 μM)	15.3 $\pm$ 2.6	0	0.12
+ L-leucine (500 μM)	13.9 $\pm$ 0.5	5	0.11
+ CaCl_2 (50 μM)	12.7 $\pm$ 1.9	14	6.45
+ CaCl_2 (500 μM)	5.1 $\pm$ 1.2	65	25.54

Isotope (1 μCi ; Radiochemical Centre) and the additions indicated were extracted with 1 ml toluene-butanol (7:3 v/v) mixture as described in Table 1. Approximate concentrations (μM) of leucine and calcium, respectively, in the final organic phase are calculated from the c.p.m., assuming that the affinity of the unlabelled compound for A23187 is identical to that of the respective radioactive isotope. Mean and range of the results from two separate extractions are given.

Table 3 Inhibition of ^3H -leucine sequestration by other amino acids and calcium

Composition of the initial water phase	Amount of ^3H -leucine in the organic phase after extraction c.p.m.	μM (mean)	% Relative inhibition (mean)
^3H -leucine (50 μM) + A23187 (5 μM)	7,102 $\pm$ 53	0.890	—
^3H -leucine (50 μM) + A23187 (5 μM) + L-leucine (50 μM)	5,678 $\pm$ 322	0.710	100
+ L-glycine (50 μM)	5,723 $\pm$ 293	0.715	97
+ L-lysine (50 μM)	5,832 $\pm$ 122	0.725	89
+ L-arginine (50 μM)	6,025 $\pm$ 585	0.750	79
+ L-aspartic acid (50 μM)	6,125 $\pm$ 535	0.765	69
+ L-glutamic acid (50 μM)	6,805 $\pm$ 545	0.850	21
+ β alanine (50 μM)	7,343 $\pm$ 42	0.915	0
+ ϵ amino caproic acid (50 μM)	7,458 $\pm$ 178	0.930	0
+ CaCl_2 (50 μM)	6,730 $\pm$ 320	0.840	26

^3H -leucine (1 μCi) and the additions as indicated were extracted with 1 ml toluene-butanol mixture as described in Table 1. Relative inhibition of ^3H -sequestration by the added compounds is calculated from the c.p.m., taking the inhibition caused by 50 μM L-leucine as 100%. Mean and range of the results from two separate extractions are given.

A23187 to sequester leucine, but it could be explained by assuming that in an aqueous medium the highly hydrophobic A23187 can acquire two different micellar forms, depending on concentration. At lower concentrations of A23187 a form with a relatively high affinity to leucine is prevalent, whereas at higher concentrations the ionophore exists mainly in another form, which can bind calcium but not leucine. The final answer to this question requires further study.

We also found that leucine is not the only amino acid with which A23187 can interact. Whereas the partitioning of ^3H -uridine and ^3H -thymidine remained practically unaffected at 0.1 to 10 μM concentrations of the ionophore (data not shown) ^{35}S -methionine was effectively transferred to the organic phase by 10 μM A23187 (Fig. 1). Furthermore, several other α amino acids were able to inhibit organic sequestration of ^3H -leucine. They showed increasing relative affinity for the ionophore in the following order: Glu $\ll$ Asp $<$ Arg $<$ Lys $<$ Gly $<$ Leu (Table 3). Other experiments have shown that cysteine and glutamine can also inhibit sequestration of ^3H -leucine. Non- α

amino acids tested, β alanine and ϵ amino caproic acid, did not show any affinity for A23187 in this system (Table 3). Again, compared with cold leucine, CaCl_2 was less effective in inhibiting ^3H -leucine sequestration (Table 3).

The relevance of these findings to the effects of A23187 on cellular physiology is difficult to assess. Although we detected EGTA-resistant induction of an enhanced rate of leucine uptake within a few hours in lymphocyte cultures incubated with the ionophore, our attempts to show direct facilitation of leucine uptake in the presence of A23187 have so far been unsuccessful. Nevertheless, the findings reported in this paper indicate that the ionophore A23187 can cause reversible organic sequestration of amino acids at concentrations as low as, or lower, than those needed to form lipid-soluble complexes with calcium, and that equimolar concentration of calcium can only slightly inhibit this complex formation. This means that apart from the well documented action of A23187 as a potent divalent cation ionophore it may have effects on cellular metabolism resulting from its affinity for amino acids. One other implication of these findings is that when A23187 is used in complex experimental conditions, caution is necessary before concluding that all the phenomena detected are simply caused by increased permeability of membranes to divalent cations.

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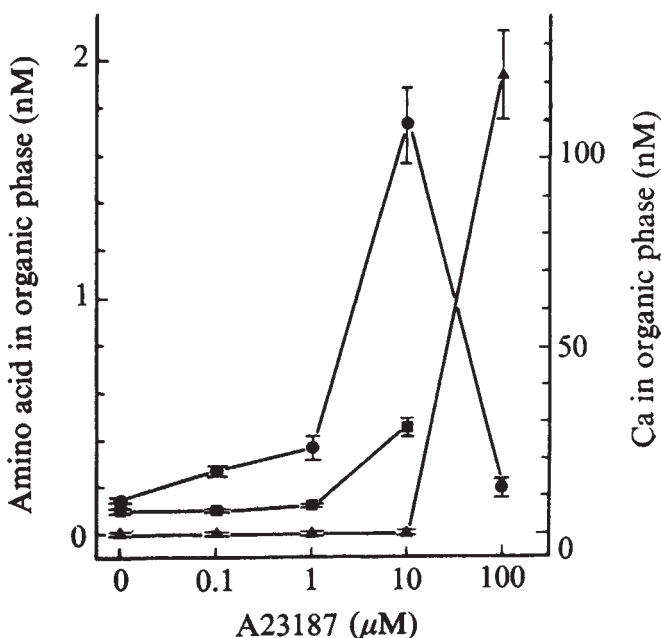
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Corrigendum

In the article "The function of phytochrome in plants growing in the natural environment" by M. G. Holmes and H. Smith (*Nature*, **254**, 512; 1975) the two arable weeds used were *Tripleurospermum martimum inodorum* (scentless mayweed) and *Chenopodium album* (fat-hen).



reviews

HERE is something to hold on to when exploring the complex terrain of animal learning. Professor Mackintosh has given us a book* that is detailed, authoritative and scholarly. In this age of symposia and multiauthored volumes, it is a rare treat to find a work of this magnitude by a single author, and even rarer to find one with an incisive and economical style. It is a large book, with more than 600 pages of text and over 2,000 references. It is also often a tough book, not only because its author does not hesitate to deal with complicated issues and material, but because his very economy of style does not cushion the reader with redundancy. It is a book to read without blinking, if not unblinkered. More than that: it is to be reread and kept as a source of relevant literature and of perceptive commentary and sophisticated analysis. Both the student and his mentor will be grateful.

The subject matter is conventional and traditional to experimental psychology—at least, it used to be traditional. It deliberately avoids the ethologists' concern with differences in creatures' capacities forced on them by particular evolutionary pressures. "Although there may be important differences in the processes of behavioural modification to be found in different animals, the study of those processes, independently of any particular concern for their specialisation in different groups, remains an important and valid branch of science. Just as the sciences of genetics, embryology, and neurophysiology were initially advanced by the search for general, fundamental principles, undertaken with subjects chosen solely for reasons of convenience, so it is reasonable to suppose that some principles will have emerged from the analysis of associative learning in dogs, rats and pigeons. It is, at any rate, this tradition, started by Thorndike in America and, more systematically, by Pavlov in Russia, that forms the subject matter of this book." (p.3).

As such, a large portion of the volume concentrates on the details and theoretical underpinning of Pavlovian and instrumental conditioning in the dog, rat and pigeon. The first really influential book that attempted to contrast and dissect these two learning

paradigms was *Conditioning and Learning*, by Hilgard and Marquis in 1940, and the comparison with the present work is of some interest. The empirical evidence now has swollen, almost, some would say, to the point of diminishing returns in a few areas of concentration. Not surprisingly, it has also become fragmented and there has resulted, Mackintosh fairly points out, "a severe failure of communication between workers in closely related areas. Thus [for example] the analysis of reinforcement in classical condition-

Careful tread on classic ground

L. Weiskrantz

ing has had little impact on theories of instrumental learning."

Mackintosh goes a long way towards re-establishing these lines of communication, and he can draw on recent advances that, somewhat overdue, have flowed from the liberation of behavioural analysis from the apparatus and paradigms that in the past have been taken almost as definitions of a particular type of learning. Thus, the discovery of autoshaping has planted a classical conditioning colony right in the middle of the instrumental conditioning reservation. Further, almost wholly new areas of interest have emerged; for example, contrast effects earn a whole chapter in Mackintosh's book whereas the term was not even known to Hilgard and Marquis, and in general the findings and experimental niceties of the operant conditioning movement are seen throughout this volume. Significantly, Hilgard and Marquis devoted hardly any space to the analysis of punishment, either theoretical or empirical, and Mackintosh's account of this important area is especially useful and succinct. Finally, it is interesting that Mackintosh—in a much stouter volume—foregoes discussion of some of the final chapter headings of Hilgard and Marquis—for example, neurophysiological mechanism and personality (both of deep concern to Pavlov)—and only touches briefly on the question of problem solving. One suspects that it

was not solely for reasons of limitations of space that this self-restraint was exercised.

It is fair to ask whether new principles have, in fact, emerged from persisting with the traditional account of associative learning in animals, as Mackintosh thinks it reasonable to suppose will have happened. Certainly, many new assertions about limited domains have emerged, but the striking strength of this book is its disavowal of easy generalisations; it takes complexity seriously, and abounds with qualifications and counter-arguments, laced with scepticism. Talking about schedules of reinforcement, a prime source of generalisations for some researchers, he says "They are frighteningly complex and not necessarily at all well suited to an elucidation of the important processes underlying instrumental behaviour. Too many variables are permitted to vary in too many ways for it to be easy to achieve any analytic understanding." At the same time, he is prepared to defend unpopular theories, such as the stimulus-substitution theory of classical conditioning. Elsewhere he is at pains to blur accepted distinctions, as when he argues that the difference between response systems of classical and instrumental conditioning is one of degree, not of kind. Overall, the case for dealing with associative learning in animals, independent of particular specialisations, is well sustained, though whatever else this volume provides, it is not a compendium of fundamental principles. But progress in this field need not suffer on that account. In some other areas of biology, such as neurophysiology, we continue to see significant advances based on the gradual accumulation of evidence combined with careful empirical and logical dissection of the controlling variables and the underlying mechanisms, rather than the all-embracing insight or sudden breakthrough.

Beyond the domain of conditioning and the excellent extensions into discrimination learning, contrast, and generalisation, one will not find much argument or space devoted to cognitive capacities commonly appealed to in studies of human learning that might also seem applicable to learning in other mammals. Even place learning, long a favourite of the animal 'cognitive' theorists, is relegated to minor importance. Learning-set phenomena are discussed only briefly. New fron-

**The Psychology of Animal Learning*. By N. J. Mackintosh. Pp. xiv+730. (Academic: London and New York, December 1974.) £8.00; \$18.50.

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tiers may not be broached, but a large and strategically located segment of the Old World has been extremely well charted. One will have to look hard for a better guide to a region that is criss-crossed with well trodden paths but still nourishes a fresh crop of delicate fruit and nettles each spring. For someone who wants to keep his feet on the ground, nettles really matter. □

Simply chemistry

Chemistry. By Linus and Peter Pauling. Pp. xi+767. (Freeman: San Francisco, 1975.) \$13.95.

THIS is essentially a new edition of the elder Pauling's highly successful *General Chemistry*. The original text has been shortened in places, largely by the omission of much of the thermodynamic material, and the scope has been expanded by the addition of an extensive and well balanced treatment of molecular biology, in which most of the important macromolecular structures are discussed. This change reflects not only some shift in the research emphasis of chemistry itself but also in the new audience for whom this book has been specifically designed, namely "students primarily interested in biology, medicine, human nutrition, and related fields". It provides thus a broad-brush picture of the whole of chemistry (inorganic, physical, nuclear, organic, and biochemistry) and its interaction with the biological sciences, at a level suitable for those first studying it at an American college.

The emphasis throughout is on the structural aspects of chemistry, even more so than in the previous editions. For many chemists the chief demerit of *Chemistry* will be its heavy reliance on electronegativity, resonance, the electroneutrality principle, and other concepts familiar to readers of *The Nature of the Chemical Bond* but less obvious in most of the current chemical literature: the authors would presumably defend their point of view as being simple, general, and particularly suited to the limited chemical sophistication of their intended readers. Others may cavil at the slight reference to modern physical techniques and organic reactions and their mechanisms.

The exposition, which is delightful with its clarity and elegance of style, will appeal to the student and arouse his or her interest with its neat and modern choice of example and illustration. There are numerous sensible, straightforward, and intriguing problems and the diagrams are excellent. I much enjoyed reading it, and so did my daughter, now in the middle of a sixth form science course. By modern standards the book is very modestly priced.

C. S. G. Phillips

From cell to cell

Cell Communication. (Wiley Series in the Dynamics of Cell Biology.) Edited by R. P. Cox. Pp. ix+262. (Wiley: New York and London, November 1974.) £11.90.

ONCE again we are victims of editorial, or perhaps publisher's pride—this book is not about all aspects of cell communication for it treats only a limited number of features of the subject. But there is one strong, important theme of the book: it contains three good reviews on the related subjects of 'tight and gap junctions', 'low-resistance pathways' and 'metabolic cooperation'. The remaining eight papers cover an assortment of subjects in which cell communication plays a greater or lesser role but no particular theme joins them or excuses the absence of so many aspects of cell communication. The diligent reader will note the lack of any mention of exo and endocytosis, cell fusion (both in fertilisation and in other contexts), cell adhesion, cell positioning and recognition phenomena, malignancy, and anything at all about plants.

The electron microscopists seem at last to have abandoned their addiction to neoclassical microanatomical latinity and the excellent article by Norton Gilula shows how *zonulae occludentes* have become tight junctions. But this is not the main virtue of his article. That lies in the beautiful electron micrographs of the specialised contacts between cells which are probably the low-resistance routes between cells and the pathways for interchange of metabolites between cells. This article summarises work from several laboratories, published in the past few years. Judson Sheridan explains the main observations carried out on low-resistance pathways and the editor and his coworkers review "Metabolic cooperation". In spite of the suggestion that appears in these three articles—that the three phenomena are but aspects of the same matter—it is still unclear as to how important the phenomena are in the real life of an animal.

The remaining articles in the book cover an assortment of subjects. Noteworthy are those by Harry Rubin on cell growth—a process in which cell communication may be unimportant—and by Albert Harris on contact inhibition. There are also papers by Fishbach on neuromuscular junction formation, Kolodny on transfer of macromolecules between cells, Cruikshank on cell interactions in the skin, Basten and Miller on the immune response, Neufeld on lysosomal diseases and Ottolenghi-Nightingale on DNA transformation in mammalian cells.

Adam Curtis

ABOUT a century ago science was in the grip of a surging interest in psychical research. In laboratories, both amateur and professional, literally hundreds of earnest scientists were conducting experiments of the most extraordinary kind—bottling ectoplasm, weighing mediums while in and out of trance, photographing materialised spirit forms, and so on. Though it may seem that such activities were at serious odds with the 19th century mechanistic view of the Universe, on second thought they seem less incongruous. Most of these pioneers were raised and educated within the ethos of Victorian Christianity which held that man was essentially an immortal spirit for whom death was a transition and not an extinction point. Furthermore, they were also steeped in the notion of the infallibility of the 19th century scientific method—everything in the Universe was fit and ready for instant laboratory investigation. Thus the weird phenomena of psychic research were no more elusive in principle than the behaviour of molecules or planets.

The rollcall of personalities involved in that phase of psychical research is breathtaking and includes Lodge, Rayleigh, Freud, Wallace, Richet, Crookes, Gladstone and J. J. Thomson, to name but a few; all came to believe that psychic research was one of the most important avenues of study for mankind, and incurred the contemptuous scorn of fellow academics as a result. Sir William Crookes for example, one of the most distinguished physicists of the century, participated actively in spiritualist seances and was so confident of his beliefs that he proudly allowed himself to be photographed arm-in-arm with a “materialised spirit form”, a diaphanously garbed but otherwise rather earthy-looking being, known as Katie King.

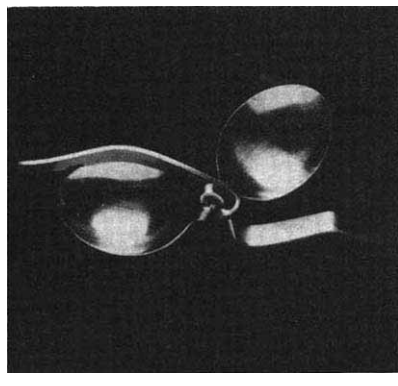
Well, a 100 years have rolled by and, in spite of the sonorous declarations of Crookes and his contemporaries and the volumes of scientific papers on psychical research, who today—apart from a few ardent spiritualists—treats bottled ectoplasm or the photographs of the Crookes-Katie King encounter as anything other than fading curiosities? Worse still, who would argue that all the industry of those gallant pioneers and the stupendous cerebral effort they invested in chasing phantoms has improved our understanding of the Universe one jot?

I have to admit that disheartening reflections of this kind drifted through my head as I scanned through John Taylor's *Superminds**. The author is a

distinguished mathematician and physicist with an international reputation in his field. He is also at this time actively preoccupied with investigating phenomena which, if not actually involving ectoplasm and spirits appearing out of floors, will seem to most scientists to be more or less in the same category. The phenomena in question involve mysterious distortions in objects such as spoons, forks and metal bars, which are alleged to take place in the general vicinity of certain children. I say “in the general vicinity of” and yet, though most of the bendings and distortions—some of them are literally grotesque, as the book's numerous illustrations reveal—require the child to stroke or

Brave but not convincing

Christopher Evans



otherwise handle the metal, others allegedly take place at distances varying from yards to miles away from the ‘bender’. As if that were not enough, the objects sometimes bend inside metal boxes, corked test tubes and even in locked cupboards.

Professor Taylor's interest in this peculiar pocket of the Universe seems to have been sparked by the notorious BBC television programme in which the flamboyant Uri Geller disintegrated a fork before an historically uncritical audience. This was followed by more closely controlled demonstrations in Taylor's laboratory at King's College, some of which unsettled him to the extent that, as he puts it, “the whole framework with which I viewed the world had suddenly been destroyed”. From here there seemed no course but to continue the investigations through to the end, whether bitter or glorious, taking as subjects some of the children whose metal bending powers sprang into being following the television demonstration.

As a result Taylor is convinced that he is tapping and probing previously unknown forces of immense potential and significance, and his *Superminds* is in part a record of his findings and in

part an attempt at providing a theoretical framework for them. For the latter he leans on hypotheses invoking electromagnetism, mainly because no other kind of theory even remotely begins to fit the facts. The soundness of this theorising is a matter for discussion by other theoretical physicists but I must raise the strong suspicion that Professor Taylor is putting the cart before the horse. Before getting drawn into the complexities of theorising it is more economic to make absolutely certain that one has not only established the phenomena beyond all doubt, but that one has also satisfied oneself that these phenomena cannot be encompassed within any existing theory.

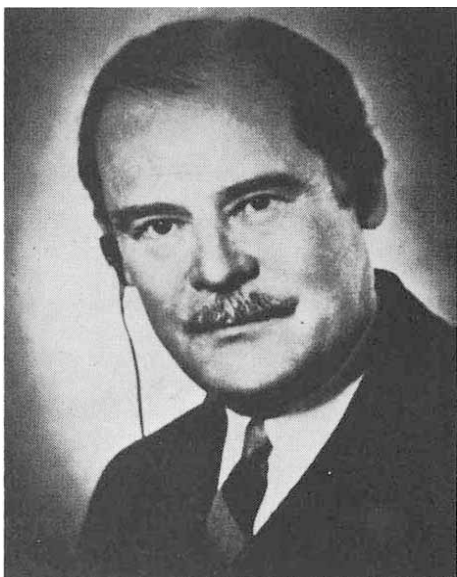
Unfortunately, though Taylor may well be convinced of this in his own mind, the material in the book will, I fear, do little to convince other scientists. My most serious objection is the ease with which he seems able to discount the possibility of fraud and deception; true, he has devised some elaborate apparatus—attaching the objects to spring balances to detect whether the benders are using physical pressure on them, and so on—but these precautions seem to me to be diversionary and indirect when more positive strategies could be used.

For example, if it is claimed that an individual can bend metal, within containers of various kinds, without touching it, then the first step should be to set up apparatus in an independent laboratory, using infrared beams to detect prying fingers, and videotape to record the metal as it distorts. Alternatively, if it is argued that laboratory conditions are inhibiting—Taylor comments, rather ingenuously, that the presence of “sceptics” tends to kill the phenomena—then all one has to do is to encapsulate metal bars in specially blown glass spheres which can be left for a given period in the homes of the ‘superminds’, and which can be inspected afterwards for signs of tampering. In fact, since writing *Superminds* Taylor has been attempting automation of his experiments, including constant videotaping, in an attempt to meet objections of this kind. The truth of the matter is, however, that if half of what is claimed on behalf of the superminds is true, then scientists and science in general deserve more solid data than he offers in this particular work. But it is still a brave book and Professor Taylor is a brave man to have written it. Alas it is the same kind of bravery, in my view, that Crookes exhibited when he took an alluring ghost by the arm and tried to convince the world of her reality. Crookes' contemporaries remained unconvinced, and so, I suspect, will Taylor's. □

**Superminds: An Enquiry into the Paranormal*. By John Taylor. Pp. 183. (Macmillan: London and Basingstoke, April 1975.) £3.95.

obituary

Ernst F. W. Alexanderson died on May 14 at Schenectady, New York, where for so many years he was a rich source of ideas and inventions for General Electric. He was 97.



General Electric, USA

Alexanderson was born at Uppsala, Sweden and studied at the University of Lund and the Royal Institute of Technology in Stockholm. Shortly after graduating he was working in Germany when he saw a paper on 'Alternating Current Phenomena' by Steinmetz and decided to go to the United States and seek employment in the author's laboratory at General Electric. He went to America in 1901, started work under Steinmetz in 1902, and within a year had established a reputation as a designer specialising in alternating current generators. At this time, just after the turn of the century, commercial wireless telegraphy was well established, but the system most generally used was the fairly crude spark transmission, which radiated a damped wave train covering an inconveniently wide frequency band and which was unsuited to any modulation more sophisticated than the on-off keying of the Morse code. If a continuous, high frequency, sinusoidal voltage could be generated it would help solve the increasingly pressing problem of interference between simultaneous transmissions from neighbouring stations, and furthermore it would be a much more promising carrier for

modulation by speech or music. In 1904, Fessenden, who had carried out successful preliminary experiments with radio telephony, brought this problem to General Electric and asked them to design a high power alternator operating at a frequency of many kHz. The task was given to Alexanderson, and on Christmas Eve, 1906, Fessenden was able to broadcast speech and music. Work on thermionic valves proceeding elsewhere in the General Electric laboratories led to efficient methods of high power modulation, and Alexanderson's alternators, producing up to 200 kW, became the basis of many commercial and military radio systems during World War I. This strongly influenced the US Government to promote the formation in 1919 of the Radio Corporation of America, combining the radio interests of General Electric with those taken over from foreign companies who were excluded from the new organisation in the national interest. Alexanderson worked full-time for General Electric and RCA until he was seventy, and as a consultant until he was nearly eighty, pouring out inventions in fields as diverse as magnetic amplification, control systems, ship propulsion, aerial design, electronic circuitry and colour television systems. He was in the great tradition of American inventor-scientists and had well over three hundred patents to his name. Of his many honours, he was probably most gratified by a decoration from King Gustav V of Sweden and by the entirely appropriate award of the Edison medal.

G. K. T. Conn, OBE, professor of physics and head of the department in the university of Exeter since 1957, died on June 4 at the age of 64

After working on infrared and Raman vibrational spectra with Sutherland's group in Cambridge, he worked both at Sheffield and Exeter on the properties of solids (metals, alloys and semiconductors)—as determined by the evaluation of the optical constants for various frequencies. This involved determining the parameters of monochromatic radiation reflected from prepared surfaces; and the frequencies involved ultraviolet, visible, infrared and far infrared, including latterly the methods of Fourier transform spectroscopy. The methods he developed with Beattie and Eaton have been adopted by a number of other workers in the

field. He was editor of *Research*, a review journal, from 1956–62, and more recently had been joint editor of a series of Essays in Physics. He was also general editor of a series of textbooks, *The Modern University Physics Series*, in current production. His terms as deputy vice-chancellor of Exeter (1967–69) involved him in a series of delicate discussions during a period of unrest besetting British universities.

Hans D. Berendes, professor of genetics at the University of Nijmegen and well known for his work on gene action and chromosome structure, died on May 25 at the age of 41.

Berendes was born in The Hague and in 1955 began his study in biology at the University of Leiden. In the advanced part of his study he started research on the salivary chromosomes of *Drosophila hydei*, which dominated his whole scientific career, and completed a cytological map of these giant chromosomes. In 1962 he was appointed as lecturer at the University of Leiden. Impressed by the work of Beermann and Clever he worked on gene action in *D. hydei* as expressed in the puffing of salivary chromosome bands. He obtained his doctors degree in 1965 for a thesis on puffing patterns in the course of the late development of larvae and pupae, and the correlation between the appearance of some puffs and a cell product. In the same period he started research on the induction of puffs by external factors, in particular heatshocks. He then took a position in Beermann's laboratory at Tübingen, where he worked on the role of ecdyson in activation of puffs, puffing patterns in different tissues, protein metabolism in puffs, differential replication of chromosomal DNA and the electron microscopic structure of the salivary chromosome. In 1968 the Universities of Geneva and Nijmegen offered him chairs in Zoology, but he preferred to return to Holland to continue his work on *D. hydei*. Important papers appeared on the ultrastructure of DNA from the giant chromosomes, on the products of certain puffs, and on the relation between the formation of puffs and synthesis of mitochondrial enzymes. The latter project promises important insight into the regulation of gene activity in higher organisms. Berendes was also a very able organiser. He participated strongly in University affairs and in the Organisation for Pure Research (ZWO).

announcements

Award

Lynn R. Sykes has been awarded the **Walter H. Bucher Medal** by the American Geophysical Union for original contributions to the basic knowledge of the Earth's crust.

Appointments

P. J. Peterson has been appointed Royal Society visiting professor attached to the School of Biological Sciences at the Universiti Sains Malaysia.

James Brown, area engineer for Africa and the Western Hemisphere, Shell International, has been appointed to the new chair of petroleum engineering at Heriot-Watt University.

The University of Sheffield has made the following appointments: **N. M. Atherton** to a personal chair in chemistry; **G. Hudson** to a personal chair in experimental haematology.

Miscellaneous

Heinz Karger Prize. Heinz Karger Memorial Foundation invites papers on: methods for early diagnosis of genetic disorders (1976); and 'molecular biology of metabolic diseases (1977). Closing dates: February 28, 1976 and 1977. For conditions and further information, contact: S. Karger AG, Arnold-Böcklin-Strasse 25, CH-4011 Basle, Switzerland.

Travel fellowships. The Winston Churchill Memorial Trust offers travel grants for 1976. This year's list of categories includes: teachers in further education, laboratory technicians, energy resources, naturalists, and conservation of coastal amenities. Further information and applications (UK citizens only): The Winston Churchill Memorial Trust, 15 Queen's Gate Terrace, London SW7 5PR, UK.

International meetings

August 21–23, **Gamete competition in plants and animals**, Lake Como, Italy (Dr D. Mulcahy, Università di Milano, Istituto di Genetica, Via Celoria, 10, 20133 Milano, Italy).

August 21–27, **Plant protection**, Moscow, USSR (Professor V. A. Lebedev, Secretary General of the Organising Committee of the Seventh International Congress of Plant Protection, Orlikov

per 1/11, Room 478, Moscow 107139, USSR).

August 22–29, **Limnology**, Winnipeg, Manitoba (Mr R. M. Raeburn, University Relations and Information Officer, The University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada).

August 23–27, **Rheology**, Gothenburg, Sweden (J. Kubat, Chairman, Organising Committee, Chalmers University of Technology, Fack, S-402 20, Goteborg 5, Sweden).

August 24–27, **Crystal growth**, Edinburgh, UK (F. W. Ainger, Allen Clark Research Centre, The Plessey Company Limited, Caswell, Towcester, Northamptonshire, UK).

August 25–29, **Atomic spectroscopy**, Clayton, Australia (Dr J. B. Willis, Secretary, Fifth International Conference on Atomic Spectroscopy, CSIRO Division of Chemical Physics, PO Box 160, Clayton, Victoria 3168, Australia).

August 25–29, **Thin films**, Budapest, Hungary (Organising Committee of ICT F3, 1325 Budapest, PO Box 76, Hungary).

August 25–29, **Cybernetics and systems**, Bucharest, Rumania (Dr J. Rose, Director General, World Organisation of General Systems and Cybernetics (WOGSC), College of Technology, Blackburn BB2 1LH, UK).

August 26–29, **Antivirals with clinical potential**, Stanford, California (Antivirals Symposium, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, California 94305).

August 27–30, **Phonon scattering in solids**, Nottingham, UK (Phonon Conference Department of Physics University of Nottingham, Nottingham NG7 2RD, UK).

August 28–30, **New first and second messengers in nervous tissues**, Brescia, Italy (Dr M. Trabucchi, Scientific secretary, Institute of Pharmacology and Pharmacognosy, University of Milan, Via Andrea Del Sarto, 21, 20129, Milan, Italy).

August 29–31, **Function and metabolism of phospholipids in nervous tissue and Biochemical and pharmacological implications of ganglioside functions**, Perugia, Italy (Professor G.

Porcellati, Chairman, Organising Committee, Istituto di Chimica Biologica, Università di Perugia, Policlinico Monteluce, C.P. 3, Succ.3, Perugia, Italy).

August 31–September 5, **Charles Lyell centenary symposium**, London, UK (J. C. Thackray, Geological Museum, Institute of Geological Sciences, Exhibition Road, London SW7 2DE, UK).

Reports and publications

Great Britain

Proceedings of the Royal Irish Academy. Vol. 75, Section A. No. 5: Circumscribing Quadrics—a New Approach to the Problem of Statistical Discrimination. By I. G. O. Muirchearthaigh. Pp.49–56. 24p. No. 6: The Nature of the Vortex Core. By P. D. McCormack. Pp.49–56. 24p. No. 6: The Nature of the Vortex Core. By P. D. McCormack. Pp.57–72. 30p. No. 7: Properties of the Variation. By P. McGill. Pp.73–78. 18p. No. 8: *SL(2,C)* and the Lorentz Group. By J. J. McMahon. Pp.79–83. 18p. Vol. 74, Section B. No. 4: Records of *Codium* Species in Ireland. By Hilda M. Parkes. Pp.125–134. 35p. No. 5: Sorption of Carbon Dioxide, and Ammonia by Zeolites Containing Univalent and Trivalent Cations. By B. Coughlan and S. Kilmartin. Pp.135–154. 45p. No. 6: The Old Red Sandstone Group of Iveragh, Co. Kerry. By J. G. Capewell. Pp.153–171. 37p. No. 7: Deep-Seated Igneous Intrusions in Co. Kerry. By D. W. Howard. Pp.173–183+plates 2 and 3. 46p. No. 8: Contributions to the Lichen Flora of South-East Ireland—I. By M. R. D. Seaward. Pp.185–205. 34p. No. 9: The Pattern of Glaciation of County Dublin. By P. G. Hoare. Pp.207–224. 48p. (Dublin: Royal Irish Academy, 1975.) [154]

The Achievement of Television. By Huw Wheldon. (BBC Lunch-Time Lectures, Ninth Series, 5.) Pp.18. (London: BBC, 1975.) [164]

The Institute of Fuel. Report and Accounts for 1974. Pp.12. (London: The Institute of Fuel, 1975.) [164]

Forestry Commission. Report on Forest Research 1974. Pp.vii+109+4 plates. £1.10 net. Fifty-Fourth Annual Report and Accounts, 1973/1974. Pp.102+6 plates. £1.05 net. (London: HMSO, 1974.) [184]

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